REMARKS

In the advisory action, the Examiner notes that the previous rejection of claims 1-4, 6, 9, 13-20, 24, 28-31, 47-50, 54 and 58-61, under the judicially created doctrine of obviousness-type double patenting in view of claims 16-17 and 26-27 of U.S. Patent No. 6,395,783, has been withdrawn.

However, claims 1-4, 6, 9, 13-20, 24, 28-31, 47-50, 54 and 58-61 stand rejected under 35 U.S.C. §103.

In response, Applicants have cancelled claims 15, 16, 30, 31, 60 and 61, and amended claims 1, 17 and 47. As a result, claims 1-4, 6, 9, 13, 14, 17-20, 24, 28, 29, 31, 47-50, 54, 58 and 59 are pending in the application. Reconsideration is respectfully requested.

I. Amendments to the Claims

Claims 1, 17, and 47 have been amended to recite that the addiction-related behavior and addiction to drugs is associated with cocaine or nicotine addiction. Support for the amendments can be found throughout the specification. In particular, examples 13 and 14 demonstrate the effectiveness of topiramate on treating addiction to nicotine and cocaine.

II. Rejection Under 35 U.S.C. §103

Claims 1-4, 6, 9, 13-20, 24, 28-31, 47-50, 54 and 58-61 have been rejected under §103(a) as being unpatentable over U.S. Patent No. 5,189,064 to Blum et al., and U.S. Patent No. 3,639,607 to Phillips, in view of U.S. Patent No. 5,332,736 to Carmosin et al.

All of the cited documents were disclosed in an information disclosure statement filed

by Applicants.

According to the Examiner, Blum discloses that GABA and GABA agonists are useful broadly in methods for the treatment of addiction or abuse of drugs such as cocaine and alcohol (i.e. reducing seizure activity during alcohol withdrawal) since GABA and GABA agonists increase GABA levels in a mammal.

The Examiner cites Phillips for allegedly disclosing that anticonvulsants are known to be useful broadly in methods of treatment of tobacco addiction.

The Examiner recognizes that neither Blum nor Phillips expressly disclose using the particular compound disclosed, i.e. topiramate, in the present application or their effective amounts in methods of treating addiction-related behavior.

Carmosin has been cited by the Examiner for disclosing that "topiramate is an anticonvulsant which is a known GABA agonist." Therefore, the Examiner contends that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to employ topiramate in methods of treating addiction-related behavior. Applicants respectfully disagree.

Response to Advisory Action

In the advisory action, the Examiner contends that "the evidence in the examples is not commensurate in scope with the claimed invention (i.e. any addiction related behavior)." In response, and in the interest of moving the application towards allowance, Applicants have amended the claims as described above to recite cocaine and nicotine addiction. Examples

13 and 14 demonstrate the effectiveness of topiramate on treating addiction to nicotine and cocaine.

The Examiner also states that "arguments of counsel cannot take the place of factually supported subjective evidence." In response, Applicants have provided below, support for the arguments made in the previous response.

"GABA Agonists" and "Anticonvulsants" are broad classes

Firstly, Applicants fail to appreciate where Carmosin states that topiramate is a GABA agonist. Regardless, the class "GABA agonist" is a broad class containing numerous compounds that act in a variety of ways, producing a variety of resulting effects. The same is true for the class "anticonvulsant."

Support for the argument that "GABA agonist" and "anticonvulsant" are broad classes, Applicants attach copies of two webpages that list anticonvulsants (www.globalrph.com/anticonvulsants.htm) and GABA agonists along with their structures (www.tocris.com/shop/catalogue.php?ItemId=4966). See Exhibits A and B, respectively.

There is More Than One GABA Receptor and Different Compounds Act on Different Receptors

Within the class "GABA agonist" there is another variable due to the fact that there is more than one GABA receptor. For example, a GABA-a and a GABA-b receptor have been identified - each affecting different pathways. Different compounds act on different GABA receptors.

Support for the contention that GABA-a and GABA-b receptors are different and

therefore affect different pathways, and that different compounds act on different GABA receptors, Applicants have attached a copy of a webpage that depicts the structure of GABA and the differences between GABA-a and GABA-b receptors. (Western Nevada Community College - tooldoc.wncc.nevada.edu/gaba.htm). See Exhibit C.

Topiramate is Not a Simple GABA Agonist and is Structuraly Unrelated to Other Anticonvulsants and GABA

It is clear from the literature that topiramate is not a simple GABA agonist. Topiramate does not bind at either the GABA or the benzodiazepine binding sites of the GABA-a receptor. Topiramate is structurally unrelated to other anticonvulsants and to GABA.

In support of the contention that topiramate is structurally unrelated to other anticonvulsants and to GABA, Applicants have provided the structures of topiramate, various anticonvulsants and GABA. See Exhibit D (package insert for topiramate (Topomax®), package insert for phenytoin (Dilantin®), package insert for valproic acid (Depakene®), See Exhibit C for the structure of GABA and Exhibit B for the structures of GABA agonists).

Preclinical data suggests that topiramate's anticonvulsant activity is promoted by several mechanisms including modification of sodium and calcium dependent action potentials, as well as by modifying the conductance at the kianate sensitive glutamate receptors.

Support for the proposed mechanism of topiramate is provided in a copy of the new drug application (NDA) filed by the manufacturer of the drug Topomax® available at www.fda.gov/medwatch/safety/2001/topomax_label.pdf. See Exhibit E. Also, a copy of a

paper comparing the very different mechanisms of topiramate and two other anti-epileptic (anti-convulsant) drugs is attached as Exhibit F.

Not All Anticonvulsants Block Addictive Drug Induced Elevations of Dopamine

Not all anticonvulsants block addictive drug induced phasic elevations of dopamine in the brain. In support thereof, Applicants have provided a paper which demonstrates that, in fact, some anticonvulsants actually increase brain dopamine levels. Thus, it is clearly not obvious that all anticonvulsants would inhibit addictive drug-induced increases in dopamine.

In the attached paper, zonisamide, an anticonvulsant, has been shown to increase dopamine to the degree that is has been suggested that it may be useful for treating Parkinson's Disease. See Exhibit G.

Not All GABA Agonists are Effective at Treating Drug Addiction

Similarly, not all GABA agonists are effective at treating drug addiction. In fact, well known GABA agonists such as phenobarbital and benzodiazepines have no claims for efficacy in the treatment of drug abuse - they are themselves drugs of abuse. Support thereof can be found on page 6 of a paper attached as Exhibit H.

Despite the anecdotal reports used to support the claims in the Phillips patent, in more than thirty (30) years since the patent was granted, anticonvulsants *per se* have never been shown to be effective in the treatment of nicotine addiction or any other addiction to drugs of abuse.

Accordingly, Applicants contend that one of ordinary skill in the art would not have reasonably expected that topiramate would have the same therapeutic usefulness in methods

of treating addiction related behavior of a mammal, as the compounds disclosed in Blum or Phillips.

As a result of Applicants invention, it has been discovered that topiramate is effective at treating addiction-related behavior associated with cocaine or nicotine addiction in a mammal. One of ordinary skill would need to conduct countless experiments with all of the anticonvulsants disclosed in Carmosin and Phillips, and all of the GABA agonists disclosed in Blum, to find compounds effective for treating addiction-related behavior associated with cocaine or nicotine addiction.

Applicants respectfully submit that the skilled artisan would not arrive at the claimed invention from reading Blum and Phillips in view of Carmosin.

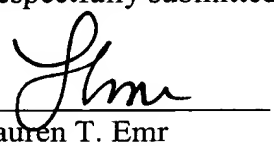
Carmosin merely adds that topiramate is an anticonvulsant. Carmosin discloses hundreds of compounds that may have anticonvulsant activity. Carmosin does not disclose or suggest that any of the anticonvulsants are suitable for treating addiction related behavior associated with cocaine or nicotine addiction, in a mammal.

Since neither Blum or Phillips disclose or suggest using topiramate to treat addiction related behavior associated with cocaine or nicotine addiction, and Carmosin merely adds that topiramate is an anticonvulsant, Applicants respectfully submit that the claimed invention is not obvious in view of the cited documents.

In light of the foregoing amendments and remarks, Applicants' respectfully submit that the application is now in condition for allowance. If the Examiner believes a telephone discussion with Applicant's representative would be of assistance, he is invited to contact the

undersigned at his convenience.

Respectfully submitted,



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Home

GlobalRph

Anticonvulsants (Adult dosing)	
carbamazepine (Tegretol ®)	Adults: initially 200 mg twice daily. Increase by 200 mg/day at weekly intervals until therapeutic levels are obtained. Usual range: 800-1200mg/day divided in 3 to 4 doses. Doses as high as 2.4 grams/day have been given.
Clonazepam (Klonopin ®):	Start 0.5 mg orally three times daily. Maximum: 20 mg/day. Supplied: [0.5,1, 2mg tabs]
diazepam (Valium ®):	Adults: Status epilepticus: I.V.: 5-10 mg every 10-20 minutes, up to 30 mg in an 8-hour period; may repeat in 2-4 hours if necessary. Anxiety/sedation/skeletal muscle relaxation: Oral: 2-10 mg 2 to 4 times/day. I.M., I.V.: 2-10 mg, may repeat in 3-4 hours if needed
ethosuximide (Zarontin ®):	Initially, 250mg orally twice a day. Maintenance 20-30mg/kg/day in divided doses twice daily. Maximum: 1.5g/day
felbamate (Felbatol ®):	Start: 400mg orally three times daily. Range: 1200 to 3600 mg daily (divided in 3 to 4 doses). Supplied: [400 , 600 mg tablets]
Fosphenytoin (Cerebyx ®):	Load: 15-20 mg/kg IM/IV. Max rate: 100-150 mg/min. Maintenance: 4 to 6 mg/kg/day divided doses every 8 to 12 hours.
gabapentin (Neurontin ®):	Start 300mg at bedtime. Increase over few days to 300 to 600mg orally three times daily. Maximum: 3600mg/day. Supplied: [100 MG, 300 MG, 400 MG CAPSULE]
levetiracetam (Keppra ®)	Adjunctive therapy in the treatment of partial-onset seizures. Start 500mg orally twice daily. Maximum: 1500 mg orally twice daily. (May adjust dose every 2 weeks). Supplied: [250, 500, 750mg tablets]
lamotrigine (Lamictal ®):	Partial/secondary generalized seizures: start: 50-100mg/day, then titrate to 100-400 mg/day in 1-2 divided doses. Possible life-threatening rash. Supplied: [25, 100, 150, 200mg tablets]
lorazepam (Ativan ®):	Adults: IV: 4 mg/dose given slowly over 2-5 minutes; may repeat in 10-15 minutes.
Phenobarbital:	Status epilepticus: Adults: 300-800 mg initially followed by 120-240 mg/dose at 20-minute intervals until seizures are controlled or a total dose of 20mg/kg. Maintenance: 1 to 3 mg/kg/day in divided doses or 50-100 mg 2 to 3 times/day.
phenytoin (Dilantin ®):	Load: 10-20 mg/kg IV. Maximum rate: 50 mg/min. Maintenance: 4-6 mg/kg/day given in 2 to 3 divided doses. Equation used to estimate the dose required to increase current level to normal range if sub-therapeutic: [0.7 x IBW x (15 - current level)]

primidone (Mysoline ®):	Start:100-125 mg orally at bedtime, increase over 10 days to 250mg orally 3 to 4 times daily
tiagabine (Gabitril ®):	Start:4 mg orally once daily, increase as needed to maximum of 56mg/day divided doses –2 to 4 times daily. Supplied: [4,12,16,20]
topiramate (Topamax ®):	Start: 50 mg at bedtime, then increase by 50mg/day (divided doses) once weekly to usual effective dose of 200mg orally twice a day.
valproic acid:	Seizures: 10-15 mg/kg/day oral / IV. Titrate to maximum of 60 mg/kg/day. Mania/migraine prophylaxis: 250 mg orally 2 to 3 times daily.

Last updated 02/17/2003 12:51:04

Search Results

There are 6 products in GABA/Glycine Receptor Compounds, GABA Receptors, GABA-A, Agonists.
back to search

Catalogue Number	Product and Pharmacological Action
0180	ZAPA sulfate
	Agonist at 'low affinity' $GABA_A$ receptor. More potent than GABA/muscimol
0181	TACA
	$GABA_A$ agonist. Also GABA-T substrate and GABA uptake inhibitor
0235	Isoguvacine hydrochloride
	Specific $GABA_A$ agonist
0289	Muscimol
	Potent $GABA_A$ agonist
0344	GABA
	Endogenous agonist
0807	THIP hydrochloride
	$GABA_A$ agonist

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0180 ZAPA sulfate

(Z)-3-[(Aminoiminomethyl)thio]prop-2-enoic acid sulfate

M.W. 244.24 $C_4H_6N_2O_2S.H_2SO_4$

Store at Room temperature
Soluble to 10 mM in water
[92138-10-8]

unit size	£	$
10 mg	39.00	59.00
50 mg	179.00	269.00

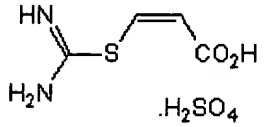
Login or register to shop

Product/Material Safety Data Sheet

Batch 4

Certificate of Analysis

Information unavaila
Please contact Custon



More potent than either GABA or muscimol as an agonist at low affinity $GABA_A$ receptors and is thus a ligand to investigate GABA receptors linked to benzodiazepine receptors. Also a $GABA_C$ receptor antag

Allan *et al* (1986) Isothiouronium compounds as γ-aminobutyric acid agonists. Br.J.Pharmacol. ***88*** 379. **Holden-Dye and Walker** (ZAPA, (Z)-3-[(aminoiminomethyl)thio]-2-propenoic acid hydrochloride, a potent agonist at GABA-receptors on the Ascaris muscle Br.J.Pharmacol. ***95*** 3. **Johnston** (1996) $GABA_C$ receptors: relatively simple transmitter-gated ion channels? TiPS ***17*** 319.

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0181 TACA

trans-4-Aminocrotonic acid

M.W. 101.1 $C_4H_7NO_2$

Store at Room temperature
Soluble to 100 mM in water
[38090-53-8]

unit size	£	$
10 mg	39.00	55.00
50 mg	155.00	229.00

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Product/Material Safety Data Sheet Batch 7

Certificate of Analysis Batch 7

The trans-isomer of CACA. Potent $GABA_A$ agonist, GABA uptake inhibitor and substrate for GABA-T. Al agonist.

Johnston *et al* (1975) *cis* and *trans*-4-Aminocrotonic acid as GABA analogues of restricted conformation. J.Neurochem. ***24*** 157. J *al* (1996) $GABA_c$ receptors: relatively simple transmitter-gated ion channels. TiPS ***17*** 319. **Chebib** *et al* (1997) Analogues of γ-ami acid (GABA) and *trans*-4-aminocrotonic acid (TACA) substituted in the 2 position as $GABA_c$ receptor antagonists. Br.J.Pharmacol.

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0235 Isoguvacine hydrochloride

1,2,3,6-Tetrahydro-4-pyridinecarboxylic acid hydrochloride

M.W. 163.6 $C_6H_9NO_2$.HCl

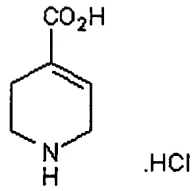
Store at Room temperature
Soluble to 100 mM in water
Purity: > 99%
[64603-90-3]

unit size	£	$
100 mg	75.00	109.00

Login or register to shop

Product/Material Safety Data Sheet Batch 21

Certificate of Analysis Batch 21



Specific GABA agonist.

Krogsgaard-Larsen *et al* (1978) Structure-activity studies on the inhibition of GABA binding to rat brain membranes by muscimol compounds. J.Neurochem. ***30*** 1377. **Krogsgaard-Larsen** *et al* (1979) Dihydromuscimol, thiomuscimol and related heterocyclic co GABA analogues. J.Neurochem. ***32*** 1717.

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0289 Muscimol

5-Aminomethyl-3-hydroxyisoxazole

M.W. 114.1 $C_4H_6N_2O_2$

Store at Room temperature

Soluble to 100 mM in 1eq. NaOH and to 100 mM in water

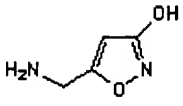
[2763-96-4]

unit size	£	$
10 mg	69.00	99.00
50 mg	285.00	429.00

Login or register to shop

Product/Material Safety Data Sheet Batch 8

Certificate of Analysis Batch 8



Potent $GABA_A$ receptor agonist and partial $GABA_C$ receptor agonist.

Johnston *et al* (1968) Central actions of ibotenic acid and muscimol. Biochem.Pharmacol. ***17*** 2488. **Akhondzadeh and Stone** (19 Induction of a novel form of hippocampal long-term depression by muscimol: involvement of $GABA_A$ but not glutamate receptors. Br.J.Pharmacol. ***115*** 527. **Johnstone** (1996) $GABA_C$ receptors: relatively simple transmitter-gated ion channels? TiPS ***17*** 319.

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0344 GABA

γ-Aminobutyric acid

M.W. 103.12 $C_4H_9NO_2$

Store at Room temperature
Soluble to 100 mM in water
[56-12-2]

unit size	£	$
1 g	15.00	19.00

Login or register to shop

Product/Material Safety Data Sheet Batch 5

Certificate of Analysis Batch 5

Endogenous inhibitory neurotransmitter.

Curtis *et al* (1970) GABA, bicuculline and central inhibition. Nature ***226*** 1222. **Decavel and van den Pol** (1990) GABA: a dominar in the hypothalamus. J.Comp.Neurol. ***302*** 1019. **Johnston** (1996) $GABA_C$ receptors: relatively simple transmitter-gated ion chann 319. **Malcangio and Bowery** (1996) GABA and its receptors in the spinal cord. TiPS ***17*** 457.

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0807 THIP hydrochloride

Gaboxadol
4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride

M.W. 176.6 $C_6H_8N_2O_2$.HCl

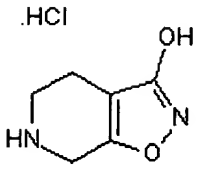
unit size	£	$
50 mg	39.00	55.00

Login or register to shop

Store at Room temperature
Soluble to 100 mM in water
Purity: > 98%
[64603-91-4]

Product/Material Safety Data Sheet Batch 7

Certificate of Analysis Batch 7



$GABA_A$ receptor agonist and $GABA_C$ receptor antagonist.

Krogsgaard-Larson (1981) Gamma-aminobutyric acid agonists, antagonists and uptake inhibitors. J.Med.Chem. ***24*** 1377. **Krogs Larson** (1983) 4,5,6,7-Tetrahydroisothiazolo[5,4-c]pyridin-3-ol and related analogues of THIP. Synthesis and biological activity. J.I ***26*** 895. **Krogsgaard-Larson** (1984) Chemistry and pharmacology of the GABA antagonists THIP (Gaboxadol) and isoguvacine. D ***9*** 597. **Johnston** (1996) $GABA_C$ receptors: relatively simple transmitter-gated ion channels? TiPS ***17*** 319.

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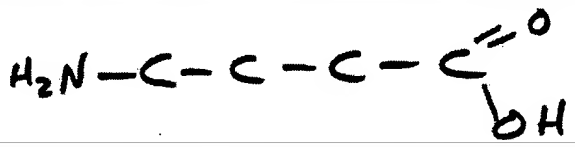
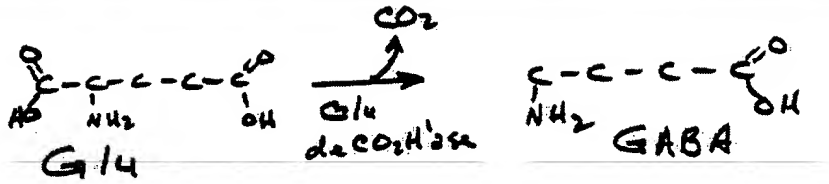
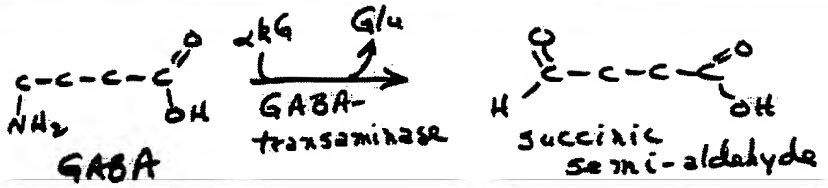
GABA

Chemical Structure of GABA: gamma-aminobutyric acid
In CNS = Primarily Inhibitory; All data coming up for GABA receptors is for peripheral receptors

Biosynthesis of GABA

Initial Degradation of GABA

$GABA_A$	Characteristic	$GABA_B$
Muscimol, isoguavicine, homotaurine	Agonists	Baclofen
Bicuculline	Antagonists	delta-aminovaleric acid
Cl^- channel OR K^+ channel	Coupled with	Ca^{2+} movement OR K^+ movement
Benzodiazepines and barbiturates	Binds also	
Enhances binding of GABA to receptors	Absence of Divalent Cations	Inhibits binding of GABA to receptors
Inhibits	Presence of Divalent Cations	Required for binding of GABA to receptors
Relaxes pial blood vessels; Decreases dopamine inhibition of PRL which results in an increased secretion of PRL from the adenohypophysis; Increases AVP release from the neurohypophysis; Pineal gland;	Location and action	Reduces HR and BP at level of atria; Increases contractility of female reproductive tract; Smooth muscle; beta-cells in pancreas; Relaxes urinary bladder;

Increases E and NE secretion from the adrenal medulla; Female reproductive tract and placenta; Contracts ileum smooth muscle; Contracts all other gut smooth muscle; Vas deferens, seminal vesicles, prostatic smooth muscle; Relaxes urinary bladder; Contracts gall bladder; Decreases stomach HCl secretion in stomach		Contracts gall bladder; Decreases stomach HCl secretion; C fibers and A-delta fibers are activated by GABA
$GABA_{generic}$: increases liver glycogenolysis with a secondary increase in BS; seems to also increase bile flow from the liver		
Further Reading:		
Erdo and Bowery, Eds: GABAergic Mechanisms in the Mammalian Periphery. (Raven Press: NY) ©1986.		Peracchia, Ed.: Handbook of Membrane Channels: Molecular and Cellular Physiology. (Academic Press: San Diego)©1994.

Muscarinic Acetylcholine Receptors (AchR's) in Brain				
M_1	M_2 ("cardiac")	M_3 ("M_2 glandular")	M_4	M_5
High affinity for pirenzipine (tricyclic antidepressant that blocks HCl secretion in stomach, without any cardiovascular effect)	High affinity for AF-DX (a benzodiazepine similar to Valium, xanax, librium, ativan)	High affinity for 4-DAMP	Limited information on these receptors	
?	Coupled with G proteins			
IP_3 with Ca^{2+} dependent cAMP (?)	G_I = cAMP	IP_3 with Ca^{2+} dependent cAMP (?)	G_I = cAMP	IP_3 with Ca^{2+} dependent cAMP (?)
IP_3	cAMP	IP_3	cAMP	IP_3
High AND low affinities for carbachol (used to treat glaucoma to reduce IOP)			Low affinity for carbachol	??????

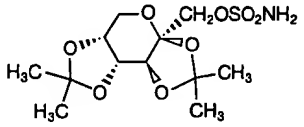
TOPAMAX®
(topiramate)
Tablets
TOPAMAX®
(topiramate capsules)
Sprinkle Capsules

Prescribing Information

DESCRIPTION

Topiramate is a sulfamate-substituted monosaccharide. TOPAMAX® (topiramate) Tablets are available as 25 mg, 50 mg, 100 mg, and 200 mg round tablets for oral administration. TOPAMAX® (topiramate capsules) Sprinkle Capsules are available as 15 mg and 25 mg sprinkle capsules for oral administration as whole capsules or opened and sprinkled onto soft food.

Topiramate is a white crystalline powder with a bitter taste. Topiramate is most soluble in alkaline solutions containing sodium hydroxide or sodium phosphate and having a pH of 9 to 10. It is freely soluble in acetone, chloroform, dimethylsulfoxide, and ethanol. The solubility in water is 9.8 mg/mL. Its saturated solution has a pH of 6.3. Topiramate has the molecular formula $C_{12}H_{21}NO_8S$ and a molecular weight of 339.37. Topiramate is designated chemically as 2,3:4,5-Di-*O*-isopropylidene-β-D-fructopyranose sulfamate and has the following structural formula:



TOPAMAX® (topiramate) Tablets contain the following inactive ingredients: lactose monohydrate, pregelatinized starch, microcrystalline cellulose, sodium starch glycolate, magnesium stearate, purified water, carnauba wax, hypromellose, titanium dioxide, polyethylene glycol, synthetic iron oxide (100 and 200 mg tablets) and polysorbate 80.

TOPAMAX® (topiramate capsules) Sprinkle Capsules contain topiramate coated beads in a hard gelatin capsule. The inactive ingredients are: sugar spheres (sucrose and starch), povidone, cellulose acetate, gelatin, silicone dioxide, sodium lauryl sulfate, titanium dioxide, and black pharmaceutical ink.

CLINICAL PHARMACOLOGY

Mechanism of Action:

The precise mechanisms by which topiramate exerts its anticonvulsant and migraine prophylaxis effects are unknown; however, preclinical studies have revealed four properties that may contribute to topiramate's efficacy for epilepsy and migraine prophylaxis. Electrophysiological and biochemical evidence suggests that topiramate, at pharmacologically relevant concentrations, blocks voltage-dependent sodium channels, augments the activity of the neurotransmitter gamma-aminobutyrate at some subtypes of the GABA-A receptor, antagonizes the AMPA/kainate subtype of the glutamate receptor, and inhibits the carbonic anhydrase enzyme, particularly isozymes II and IV.

Pharmacodynamics:

Topiramate has anticonvulsant activity in rat and mouse maximal electroshock seizure (MES) tests. Topiramate is only weakly effective in blocking clonic seizures induced by the $GABA_A$ receptor antagonist, pentylenetetrazole. Topiramate is also effective in rodent models of epilepsy, which include tonic and absence-like seizures in the spontaneous epileptic rat (SER) and tonic and clonic seizures induced in rats by kindling of the amygdala or by global ischemia.

Pharmacokinetics:

The sprinkle formulation is bioequivalent to the immediate release tablet formulation and, therefore, may be substituted as a therapeutic equivalent.

Absorption of topiramate is rapid, with peak plasma concentrations occurring at approximately 2 hours following a 400 mg oral dose. The relative bioavailability of topiramate from the tablet formulation is about 80% compared to a solution. The bioavailability of topiramate is not affected by food.

The pharmacokinetics of topiramate are linear with dose proportional increases in plasma concentration over the dose range studied (200 to 800 mg/day). The mean plasma elimination half-life is 21 hours after single or multiple doses. Steady state is thus reached in about 4 days in patients with normal renal function. Topiramate is 15-41% bound to human plasma proteins over the blood concentration range of 0.5-250 µg/mL. The fraction bound decreased as blood concentration increased.

Carbamazepine and phenytoin do not alter the binding of topiramate. Sodium valproate, at 500 µg/ml (a concentration 5-10 times higher than considered therapeutic for valproate) decreased the protein binding of topiramate from 23% to 13%. Topiramate does not influence the binding of sodium valproate.

Metabolism and Excretion:

Topiramate is not extensively metabolized and is primarily eliminated unchanged in the urine (approximately 70% of an administered dose). Six metabolites have been identified in humans, none of which constitutes more than 5% of an administered dose. The metabolites are formed via hydroxylation, hydrolysis, and glucuronidation. There is evidence of renal tubular reabsorption of topiramate. In rats, given probenecid to inhibit tubular reabsorption, along with topiramate, a significant increase in renal clearance of topiramate was observed. This interaction has not been evaluated in humans. Overall, oral plasma clearance (CL/F) is approximately 20 to 30 mL/min in humans following oral administration.

Pharmacokinetic Interactions (see also Drug Interactions):

Antiepileptic Drugs

Potential interactions between topiramate and standard AEDs were assessed in controlled clinical pharmacokinetic studies in patients with epilepsy. The effect of these interactions on mean plasma AUCs are summarized under **PRECAUTIONS (Table 3).**

Special Populations:

Renal Impairment:

The clearance of topiramate was reduced by 42% in moderately renally impaired (creatinine clearance 30-69 mL/min/1.73m^2) and by 54% in severely renally impaired subjects (creatinine clearance <30 mL/min/1.73m^2) compared to normal renal function subjects (creatinine clearance >70 mL/min/1.73m^2). Since topiramate is presumed to undergo significant tubular reabsorption, it is uncertain whether this experience can be generalized to all situations of renal impairment. It is conceivable that some forms of renal disease could differentially affect glomerular filtration rate and tubular reabsorption resulting in a clearance of topiramate not predicted by creatinine clearance. In general, however, use of one-half the usual starting and maintenance dose is recommended in patients with moderate or severe renal impairment (see **PRECAUTIONS: General** and **DOSAGE AND ADMINISTRATION**).

Hemodialysis:

Topiramate is cleared by hemodialysis. Using a high efficiency, counterflow, single pass-dialysate hemodialysis procedure, topiramate dialysis clearance was 120 mL/min with blood flow through the dialyzer at 400 mL/min. This high clearance (compared to 20-30 mL/min total oral clearance in healthy adults) will remove a clinically significant amount of topiramate from the patient over the hemodialysis treatment period. Therefore, a supplemental dose may be required (see **DOSAGE AND ADMINISTRATION**).

Hepatic Impairment:

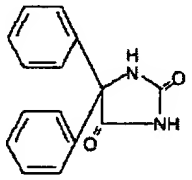
In hepatically impaired subjects, the clearance of topiramate may be decreased; the mechanism underlying the decrease is not well understood.

Dilantin-125®

(Phenytoin Oral Suspension, USP)

DESCRIPTION

Dilantin (phenytoin) is related to the barbiturates in chemical structure, but has a five-membered ring. The chemical name is 5,5-diphenyl-2,4 imidazolidinedione, having the following structural formula:



Each teaspoonful of suspension contains 125 mg of phenytoin, USP with a maximum alcohol content not greater than 0.6 percent. Also contains carboxymethylcellulose sodium, USP; citric acid, anhydrous, USP; flavors; glycerin, USP; magnesium aluminum silicate, NF; polysorbate 40, NF; purified water, USP; sodium benzoate, NF; sucrose, NF; vanillin, NF; and FD&C yellow No. 6.

CLINICAL PHARMACOLOGY

Phenytoin is an antiepileptic drug which can be useful in the treatment of epilepsy. The primary site of action appears to be *the motor cortex* where spread of seizure activity is inhibited. Possibly by promoting sodium efflux from neurons, phenytoin tends to *stabilize* the threshold against hyperexcitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient. This includes the reduction of posttetanic potentiation at synapses. Loss of posttetanic potentiation prevents cortical seizure foci from detonating adjacent cortical areas. Phenytoin reduces the maximal activity of brain stem centers responsible for the tonic phase of tonic-clonic (grand mal) seizures.

The plasma half-life in man after oral administration of phenytoin averages 22 hours, with a range of 7 to 42 hours. Steady-state therapeutic levels are achieved at least 7 to 10 days (5-7 half-lives) after initiation of therapy with recommended doses of 300 mg/day.

When serum level determinations are necessary, they should be obtained at least 5-7 half-lives after treatment initiation, dosage change, or addition or subtraction of another drug to the regimen so that equilibrium or steady-state will have been achieved. Trough levels provide information about clinically effective serum level range and confirm patient compliance and are obtained just prior to the patient's next scheduled dose. Peak levels indicate an individual's threshold for emergence of dose-related side effects and are obtained at the time of expected peak concentration. For Dilantin-125 Suspension peak levels occur 1½-3 hours after administration.

Optimum control without clinical signs of toxicity occurs more often with serum levels between 10 and 20 mcg/mL, although some mild cases of tonic-clonic (grand mal) epilepsy may be controlled with lower serum levels of phenytoin.

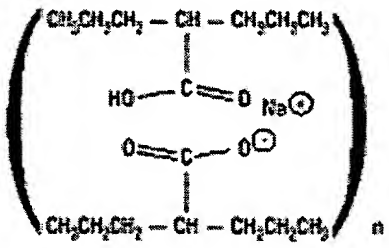
In most patients maintained at a steady dosage, stable phenytoin serum levels are achieved. There may be wide interpatient variability in phenytoin serum levels with equivalent dosages. Patients with unusually low levels may be noncompliant or hypermetabolizers of phenytoin. Unusually high levels result from liver disease, congenital enzyme deficiency, or drug interactions which result in metabolic interference. The patient with large variations in phenytoin plasma levels, despite standard doses, presents a difficult clinical problem. Serum level determinations in such patients may be particularly helpful. As phenytoin is highly protein bound, free phenytoin levels may be altered in patients whose protein binding characteristics differ from normal.

DEPAKENE®
VALPROIC ACID
CAPSULES AND SYRUP
R_x only

BOX WARNING:
HEPATIC FAILURE RESULTING IN FATALITIES HAS OCCURRED IN PATIENTS RECEIVING VALPROIC ACIDAND ITS DERIVATIVES. EXPERIENCE HAS INDICATED THAT CHILDREN UNDER THE AGE OF TWO YEARS ARE AT A CONSIDERABLY INCREASED RISK OF DEVELOPING FATAL HEPATOTOXICITY, ESPECIALLYTHOSE ON MULTIPLE ANTICONVULSANTS, THOSE WITH CONGENITAL METABOLIC DISORDERS, THOSE WITH SEVERE SEIZURE DISORDERS ACCOMPANIED BY MENTAL RETARDATION, AND THOSE WITH ORGANIC BRAIN DISEASE. WHEN DEPAKOTE IS USED IN THIS PATIENT GROUP, IT SHOULD BE USED WITH EXTREME CAUTION AND AS A SOLE AGENT. THE BENEFITS OF THERAPY SHOULD BE WEIGHED AGAINST THE RISKS. ABOVE THIS AGE GROUP, EXPERIENCE IN EPILEPSY HAS INDICATED THAT THE INCIDENCE OF FATAL HEPATOTOXICITY DECREASES CONSIDERABLY IN PROGRESSIVELY OLDER PATIENT GROUPS. THESE INCIDENTS USUALLY HAVE OCCURRED DURING THE FIRST SIX MONTHS OF TREATMENT. SERIOUS OR FATAL HEPATOTOXICITY MAY BE PRECEDED BY NON-SPECIFIC SYMPTOMS SUCH AS MALAISE, WEAKNESS, LETHARGY, FACIAL EDEMA, ANOREXIA, AND VOMITING. IN PATIENTS WITH EPILEPSY, A LOSS OF SEIZURE CONTROL MAY ALSO OCCUR. PATIENTS SHOULD BE MONITORED CLOSELY FOR APPEARANCE OF THESE SYMPTOMS. LIVER FUNCTION TESTS SHOULD BE PERFORMED PRIOR TO THERAPYAND AT FREQUENT INTERVALS THEREAFTER, ESPECIALLY DURING THE FIRST SIX MONTHS.

TERATOGENICITY:
VALPROATE CAN PRODUCE TERATOGENIC EFFECTS SUCH AS NEURAL TUBE DEFECTS (E.G., SPINA BIFIDA), ACCORDINGLY, THE USE OF DEPAKOTE TABLETS IN WOMEN OF CHILDBEARING POTENTIAL REQUIRES THAT THE BENEFITS OF ITS USE BE WEIGHED AGAINST THE RISK OF INJURY TO THE FETUS. THIS IS ESPECIALLY IMPORTANT WHEN THE TREATMENT OF A SPONTANEOUSLY REVERSIBLE CONDITION NOT ORDINARILY ASSOCIATED WITH PERMANENT INJURY OR RISK OF DEATH (E.G., MIGRAINE) IS CONTEMPLATED. SEE WARNINGS, INFORMATION FOR PATIENTS.

DESCRIPTION
DEPAKENE (valproic acid) is a carboxylic acid designated as 2-propylpentanoic acid. It is also known as dipropylacetic acid. Valproic acid has the following structure:



Valproic acid (pKa 4.8) has a molecular weight of 144 and occurs as a colorless liquid with a characteristic odor. It is slightly soluble in water (1.3 mg/mL) and very soluble in organic solvents.

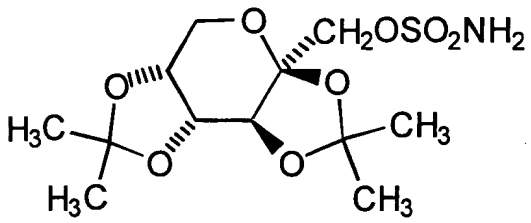
DEPAKENE capsules and syrup are antiepileptics for oral administration. Each soft elastic capsule contains 250 mg valproic acid. The syrup contains the equivalent of 250 mg valproic acid per 5 mL as the sodium salt.

Inactive Ingredients
250 mg capsules: corn oil, FD&C Yellow No. 6, gelatin, glycerin, iron oxide, methylparaben, propylparaben, and titanium dioxide.

DESCRIPTION

Topiramate is a sulfamate-substituted monosaccharide that is intended for use as an antiepileptic drug. TOPAMAX® (topiramate) Tablets are available as 25 mg, 100 mg, and 200 mg round tablets for oral administration. TOPAMAX® (topiramate capsules) Sprinkle Capsules are available as 15 mg and 25 mg sprinkle capsules for oral administration as whole capsules or opened and sprinkled onto soft food.

Topiramate is a white crystalline powder with a bitter taste. Topiramate is most soluble in alkaline solutions containing sodium hydroxide or sodium phosphate and having a pH of 9 to 10. It is freely soluble in acetone, chloroform, dimethylsulfoxide, and ethanol. The solubility in water is 9.8 mg/mL. Its saturated solution has a pH of 6.3. Topiramate has the molecular formula $C_{12}H_{21}NO_8S$ and a molecular weight of 339.37. Topiramate is designated chemically as 2,3:4,5-Di-*O*-isopropylidene-β-D-fructopyranose sulfamate and has the following structural formula:



TOPAMAX® (topiramate) Tablets contain the following inactive ingredients: lactose monohydrate, pregelatinized starch, microcrystalline cellulose, sodium starch glycolate, magnesium stearate, purified water, carnauba wax, hydroxypropyl methylcellulose, titanium dioxide, polyethylene glycol, synthetic iron oxide (100 and 200 mg tablets) and polysorbate 80.

TOPAMAX® (topiramate capsules) Sprinkle Capsules contain topiramate coated beads in a hard gelatin capsule. The inactive ingredients are: sugar spheres (sucrose and starch), povidone, cellulose acetate, gelatin, silicone dioxide, sodium lauryl sulfate, titanium dioxide, and black pharmaceutical ink.

CLINICAL PHARMACOLOGY

Mechanism of Action:

The precise mechanism by which topiramate exerts its antiseizure effect is unknown; however, electrophysiological and biochemical studies of the effects of topiramate on cultured neurons have revealed three properties that may contribute to topiramate's antiepileptic efficacy. First, action potentials elicited repetitively by a sustained depolarization of the neurons are blocked by topiramate in a time-dependent manner, suggestive of a state-dependent sodium channel blocking action. Second, topiramate increases the frequency at which Ƴ-aminobutyrate (GABA) activates $GABA_A$ receptors, and enhances the ability of GABA to induce a flux of chloride ions into neurons, suggesting that topiramate potentiates the activity of this inhibitory neurotransmitter. This effect was not blocked by flumazenil, a benzodiazepine antagonist, nor did topiramate increase the duration of the channel open time, differentiating topiramate from barbiturates that modulate $GABA_A$ receptors. Third, topiramate antagonizes the ability of kainate to activate the kainate/AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; non-NMDA) subtype of excitatory amino acid (glutamate) receptor, but has no apparent effect on the activity of N-methyl-D-aspartate (NMDA) at the NMDA receptor subtype. These effects of topiramate are concentration-dependent within the range of 1 μM to 200 μM.

Topiramate also inhibits some isoenzymes of carbonic anhydrase (CA-II and CA-IV). This pharmacologic effect is generally weaker than that of acetazolamide, a known carbonic anhydrase inhibitor, and is not thought to be a major contributing factor to topiramate's antiepileptic activity.

Pharmacodynamics:

Topiramate has anticonvulsant activity in rat and mouse maximal electroshock seizure (MES) tests. Topiramate is only weakly effective in blocking clonic seizures induced by the $GABA_A$ receptor antagonist, pentylenetetrazole. Topiramate is also effective in rodent models of epilepsy, which include tonic and absence-like seizures in the spontaneous epileptic rat (SER) and tonic and clonic seizures induced in rats by kindling of the amygdala or by global ischemia.

Pharmacokinetics:

The sprinkle formulation is bioequivalent to the immediate release tablet formulation and, therefore, may be substituted as a therapeutic equivalent.

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Novel Mechanisms of Action of Three Antiepileptic Drugs, Vigabatrin, Tiagabine, and Topiramate*

Mikael Ängehagen,[1] Elinor Ben-Menachem,[1] Lars Rönnbäck,[1] and Elisabeth Hansson[1,2]

(Accepted August 19, 2002)

Epilepsy, a functional disturbance of the CNS and induced by abnormal electrical discharges, manifests by recurrent seizures. Although new antiepileptic drugs have been developed during recent years, still more than one third of patients with epilepsy are refractory to treatment. Therefore, the search for new mechanisms that can regulate cellular excitability are of utmost importance. Three currently available drugs are of special interest because they have novel mechanisms of action and are especially effective for partial onset seizures. Vigabatrin is a selective and irreversible GABA-transaminase inhibitor that greatly increases whole-brain levels of GABA. Tiagabine is a potent inhibitor of GABA uptake into neurons and glial cells. Topiramate is considered to produce its antiepileptic effect through several mechanisms, including modification of Na^+-and/or Ca^{2+}-dependent action potentials, enhancement of GABA-mediated Cl^- fluxes into neurons, and inhibition of kainate-mediated conductance at glutamate receptors of the AMPA/kainate type. This review will discuss these mechanisms of action at the cellular and molecular levels.

KEY WORDS: Epilepsy; GABA; glutamate; antiepileptic drug; vigabatrin; tiagabin; topiramate.

INTRODUCTION

Epilepsy is one of the oldest recorded neurological disorders, with at least 3000 years of written history. Modern treatment of epilepsy began in 1857 when Sir Charles Locock discovered bromide. In developed countries, the age-adjusted incidence of recurrent unprovoked seizure ranges from 24/1000 to 53/1000 persons per year, and it is consistent across geographical areas. The age-adjusted prevalence rate varies from 4 to 8 per 1000 (1). The age-specific incidence in industrialized countries shows an U-shaped curve: incidence is high in the youngest age-group (highest in the first months of life), lowest during adult life, and increases again in the elderly (steeply after 70 years) (1).

Epilepsies are chronic central nervous system (CNS) functional disturbances that manifest by recurrent seizures and are induced by abnormal electrical discharges within the CNS. In spite of the rapid development of new antiepileptic drugs over the past 20 years, approximately 30% of patients are still refractory to all types of treatments such as medication, epilepsy surgery, and vagus nerve stimulation (2). Thus, the search for new mechanisms of action that can regulate cellular excitability are vitally needed. New techniques to elucidate cellular regulation of excitatory and inhibitory mechanisms as well as cell death can lead to the discovery of more effective drugs. During the last two decades there are three drugs currently

* Special issue dedicated to Dr. Anders Hamberger.

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available that stand out as having novel mechanisms of action, especially for partial onset seizures. This review will discuss these mechanisms with the hope that the study of the mechanisms of action of both old and newly developed drugs will further the understanding of the cellular and molecular mechanisms of epilepsy.

DEVELOPMENT AND EVOLUTION OF SEIZURES

The equilibrium in communication between neurons is regulated by excitatory and inhibitory mechanisms. Both enhancement of excitatory and impairment of inhibitory mechanisms can disturb this equilibrium and result in a seizure. The communication between neurons is mediated by action potentials and signal transduction by synaptic transmission. On the synaptic level, excitatory synaptic transmission mediated mostly by glutamate and other excitatory amino acids is balanced by the inhibitory neurotransmission predominantly mediated by gamma aminobutyric acid (GABA) and glycine (3–5). Consequently, drugs that augment inhibitory neurotransmission or reduce excitatory neurotransmission prove to be protective in many seizure models. At the cellular level, depolarizing inward currents, such as Na^+ and Ca^{2+} ionic currents, are balanced by repolarizing outward currents such as voltage and Ca^{2+}-dependent K^+ ion currents. In addition, Ca^{2+}-dependent Cl^- ion currents as well as Ca^{2+}-dependent cation currents are involved in the regulation of neuronal excitability. The behavior of these ion currents varies because different subunits can combine to form the native ionic channel. Moreover, the channels also can be modulated by intracellular protein kinases and phosphatases (6). Therefore, disruption of Na^+ channel activation or a decrease in K^+ channels leads to incomplete membrane repolarization, resulting in hyperexcitability and abnormal electrical discharges. GABA is the most common inhibitory neurotransmitter. It hyperpolarizes the cell membrane, causing inhibitory postsynaptic potentials by opening Cl^- channels when it is bound to $GABA_A$ receptors and by opening K^+ channels by second messenger mechanisms when it is bound to $GABA_B$ receptors. Epileptiform discharges are ultimately expressed by augmentation of glutamate-mediated excitatory, or impairment of GABA-mediated inhibitory, mechanisms. The transmission and synchronization of the resulting discharge is largely dependent upon neuronal system instability at the hippocampus level, allowing discharge propagation and prolongation. This excess of glutamate, released from neuronal synapses during seizures, can result in overstimulation of glutamate receptors, thus leading to a massive Ca^{2+} influx into the cells, with subsequent Ca^{2+}-activated intracellular signaling pathways (7,8). Further downstream in these intracellular signal transduction schemes, processes leading to cell damage and even cell death can occur (9,10).

SEIZURE AND NEUROPROTECTION

Synaptic overactivation leads to the excessive release of glutamate. Glutamate activates a number of postsynaptic cell membrane receptors that produce an influx of Ca^{2+} ions. This excess of Ca^{2+} then activates intracellular Ca^{2+}-dependent signaling cascades that can lead to neuronal cell death (11,12). Glutamate is the major excitatory neurotransmitter in the CNS. Glutamate taken up by astrocytes is neutralized by conversion to glutamine. Glutamine can then be released for reuptake by neurons and used to regenerate glutamate for neurotransmission. Glutamate is a precursor to GABA, as well as a precursor for glutathione (13), which is known to protect cells against reactive oxygen species (14). Calcium ions are intracellular messengers governing a large number of cellular functions, such as cell growth and differentiation, membrane excitability, exocytosis, and synaptic activity. However, an excess of Ca^{2+} loading, exceeding the capacity of Ca^{2+} regulatory mechanisms, may activate Ca^{2+}-dependent processes such as enzymes (14), energy depletion (15,16), and the formation of reactive hydrogen species (17–19). When overactivated, such processes can damage neurons or lead to the formation of toxic reaction products, which cause cell death. Seizure-induced cell death most likely involves an excitotoxic mechanism activated by the *N*-methyl-D-aspartate (NMDA) or kainate/AMPA subtype of glutamate receptors (9,20). Free radical production has also been linked to seizure-induced cell death (10). To protect neurons from glutamate-induced excitotoxicity, it is essential to prevent excessive Ca^{2+} influx into the cells or provide substances that could prevent secondary effects of such an increase of intracellular Ca^{2+}. One way to achieve this is to use antiepileptic drugs with a broad mechanism of action, for example, those that involve an antagonistic effect on excitatory neuronal receptors and an agonistic effect on inhibitory receptors.

MECHANISMS OF ACTION OF THREE ANTIEPILEPTIC DRUGS: VIGABATRIN, TIAGABINE AND TOPIRAMATE

Vigabatrin

Although it was known that decreases in brain GABA caused convulsions in animals, and drugs that increase GABA functions could have an anticonvulsant effect, the first proposal that GABA might be an inhibitory neurotransmitter came from Elliot and van Gelder (21) in 1958. Several compounds have since been successfully developed for the treatment of epilepsy that effect $GABA_A$-mediated inhibition. Vigabatrin (γ-vinyl-GABA), however, is unique because it is the only selective, irreversible GABA-transaminase (GABA-T) inhibitor that greatly increases whole-brain levels of GABA, presumably making it more available to its receptor site (Fig. 1).

Vigabatrin is now available worldwide for use as an anticonvulsant except in the United States. It has consistently been shown to be effective in the treatment of partial seizures and infantile spasms. Vigabatrin has not been approved for use by the U.S. Food and Drug Administration (FDA); it received an unapprovable letter because of the discovery of visual peripheral field defects occurring in more than 30% of patients. Although not used as a front-line antiepileptic drug (AED), many patients continue to take the drug and receive benefit from it.

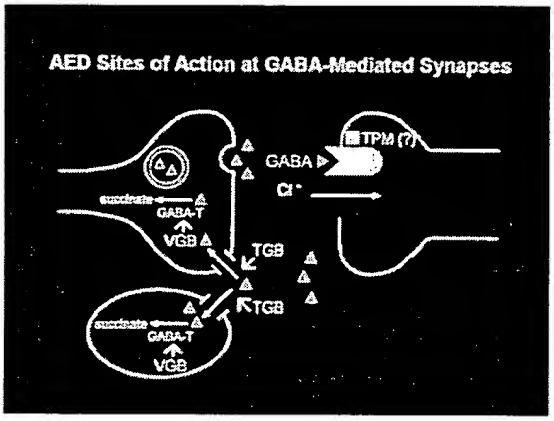

Fig. 1. Schematic drawing demonstrating effects mediated by vigabatrin (VGB), tiagabin (TGB), and topiramate (TPM) at an inhibitory synapse with GABA as the transmitter. Presynaptic terminal to the left in the figure and postsynaptic terminal to the right with a GABA receptor and a Cl^- channel. The circle below the presynaptic terminal represents a glial cell. TGB inhibits GABA uptake into both presynaptic terminal and glial cell, whereas VGB inhibits GABA-transaminase (GABA-T), the enzyme that converts GABA to succinate. The result of both drugs will be an increased extracellular GABA content. Furthermore, TPM has been shown to enhance GABA-mediated Cl^- fluxes into neurons.

Vigabatrin resembles GABA with the exception that there is a vinyl appendage. The major antiepileptic effects are determined by the half-life of the enzyme, GABA-T, rather than by the drug itself. This can be explained because GABA-T, which is the target enzyme of vigabatrin, has a much longer half-life than the drug itself (22).

Vigabatrin causes specific effects in the brain. The brain contents of GABA and GABA-T were studied following injections of 1500 mg/kg of vigabatrin in mice. By 4 hr, brain GABA had increased five-fold, whereas GABA-T activity declined sharply. Recovery to 60% of baseline concentrations of GABA-T occurred after 5 days (22).

Vigabatrin is inactive in the many animal models of epilepsy-like maximal electroshock (MES), bicuculline-induced seizures (GABA antagonist), and pentylenetetrazol-induced seizures (23). However, an intravenous injection provided seizure protection after strychnine-induced tonic seizures (24), isoniazid-induced generalized seizures (24), audiogenic seizures in mice (25), photic induced seizures in the baboon (26), and amygdala-kindled seizures in the rat (24).

Studies in Humans. The observation of changes in CSF GABA and other neurotransmitters in humans has been an important method to study the effects of vigabatrin on GABA and other neurotransmitters and amino acids in the brain. The first study to investigate the relationship between vigabatrin and GABA in the cerebrospinal fluid (CSF) was carried out by Grove and coworkers (27). Patients with varied neurological conditions were given 0.5, 1, 2, or 6 g daily of vigabatrin for 3 days. Free and total GABA, beta-alanine, homocarnosine, and vigabatrin increased in a dose-responsive manner except at the dose of 0.5 g/day, where no changes in the parameters studied were noted. Schechter and co-workers (28) studied 10 patients given 0.5 g vigabatrin twice daily followed by 1g twice daily for 2 weeks and then 2 weeks on placebo. When measured at the end of the treatment period there were no changes from baseline in homovanillic acid (HVA) the metabolite of dopamine, and 5-hydroxyindoleacetic acid (5-HIAA) the metabolite of serotonin, but dose-related increases were seen in free and total GABA and homocarnosine. At the end of the placebo period the levels of GABA and homocarnosine had declined to baseline levels.

No changes in the CSF concentrations of acetylcholine, somatostatin, beta-endorphins, prolactin, cAMP, or cGMP, were observed during chronic treatment (29). No consistent changes have been found in amino acids, HVA, or 5-HIAA with chronic treatment of vigabatrin at 50 mg/kg up to 3½ years, both in tissue and CSF (30). In a single-dose study, however, HVA and 5-HIAA concentrations increased initially up to 100%, but returned to the baseline level or slightly below baseline level after 1 month of treatment (31).

At a dose of 50 mg/kg, vigabatrin caused a 200%–300% increase in GABA in the CSF and brain tissues (31,32). A reduction of vigabatrin dose from 3 g/day to 1.5 g/day resulted in a proportional decrease of GABA levels in the CSF (33). Dose and percentage increase in CSF GABA concentrations shows a linear relationship, but the relationship between dose and efficacy appears more complex and may depend on the nature of the epilepsy (32,34). Kälviäinen and coworkers (35) have suggested that responders to vigabatrin monotherapy have higher glutamate levels (increased 14%) in the CSF before receiving the drug than the patients that did not respond. Recently NMR spectroscopy in patients treated with vigabatrin in addition to conventional AEDs have confirmed the observations seen using GABA analysis from the CSF (36). However, the authors also found increased levels of glutamine and corresponding decreased levels of glutamate by 9% compared to other patients on conventional AED therapy alone. In another study, CSF GABA increases were correlated with decreases in regional cerebral metabolism as seen in PET studies (36,37).

The effects of vigabatrin are also seen in the blood GABA and platelet GABA-T levels. Administration of vigabatrin causes a marked reduction in platelet GABA-T at therapeutic doses of 2–3 g/day. It appears that 2 g/day will maximally inhibit platelet GABA-T, with mean enzyme inhibition at approximately 70% (38). The concentration of plasma vigabatrin is almost 10-fold that seen in the CSF, and because platelets cannot regenerate GABA-T, the effect of vigabatrin on this test system will also be influenced by platelet regeneration.

Tiagabine

Tiagabine hydrochloride (R-N-(4,4-di(3-methylthien-2-yl)-but-3-enyl-nipecotic acid hydrochloride) (TGB) is an effective AED that is used primarily for the treatment of patients with partial onset seizures. It is available in most countries, including the United States. Its use for primary generalized seizures is limited, because it tends to aggravate certain seizure types, such as myoclonic jerks and absences. It is indeed interesting that both tiagabine and vigabatrin are narrow-spectrum AEDs but are extremely effective for partial onset-seizure types.

Tiagabine is a potent inhibitor of GABA uptake into neurons and glial cells. It was discovered by the pharmaceutical company Novo Nordisk in Denmark and is a lipophilic derivative of R-nipecotic acid. TGB is a highly selective and potent inhibitor of GABA transporter 1, (GAT-1). Therefore, tiagabine is involved in modulating GABA levels, especially in areas such as the hippocampus and thalamus. When given through a microdialysis probe (39), tiagabine increased GABA levels in the hippocampus by 645% and 409% in the thalamus. On the other hand, in patients receiving tiagabine, CSF levels of GABA were not increased (unpublished data, Ben-Menachem, 1996) indicating that the modulation of GABA is probably at the synaptic level and not, as with vigabatrin, obvious in the whole CNS. This is probably why no changes in peripheral vision have been observed with tiagabine in spite of intensive investigations (40,41). Thus, tiagabine, which has similar efficacy to vigabatrin in the control of partial seizures (42), has a more specific and direct effect on uptake of GABA into neurons and glial cells.

Topiramate

Topiramate [2,3:4,5-*bis*-*O*-(1-methylethylidene)β-D-fructo-pyranose sulfamate] (TPM) is a sulphamate-substituted monosaccharide derived from D-fructose (43). It is structurally unrelated to other AEDs. TPM is used as monotherapy or as add-on to other AEDs for the treatment of partial seizures, for seizures associated with Lennox-Gastaut syndrome, and for primary generalized tonic-clonic seizures (44–46).

Mechanisms of Action (figure 2). In vitro evidence suggests that TPM affects neuronal activity and produces its antiepileptic effect by several mechanisms, including modification of Na^+- and/or Ca^{2+}-dependent action potentials, enhancement of GABA activity, and inhibition of kainate (KA)-mediated conductance at glutamate receptors of the KA type (47–51). TPM has a week inhibitory effect on some carbonic anhydrase isoenzymes (52).

Na^+ Channels. Electrophysiological studies have demonstrated TPM's activity on cultured rat hippocampal neurons. Studies have revealed that TPM suppresses the voltage-sensitive Na^+ channel (42,47) mechanism of action shared with other AEDs such as phenytoin, carbamazepine, and lamotrigine (53).

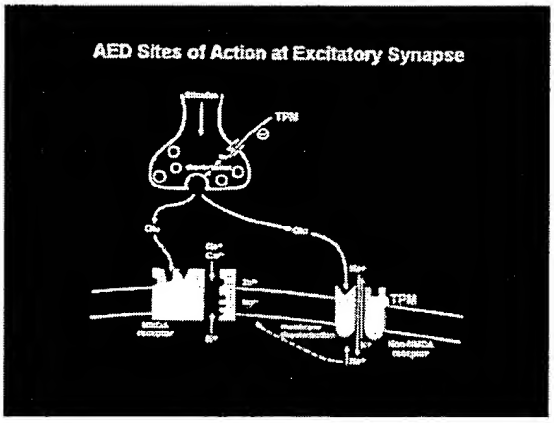

Fig. 2. Schematic drawing illustrating interaction of topiramate (TPM) with an excitatory synapse using glutamate (Glu) as transmitter. TPM inhibits presynaptic Na^+ channels (top in the figure). Until now TPM has not been shown to interact with NMDA-receptors, but with the non-NMDA receptors, especially of the kainate type. TPM has been shown to inhibit the activity of this kainate receptor in a concentration-dependent manner. The blocking of kainate evoked potentials reduces neuronal excitability and could contribute to the antiepileptic effect of the TPM.

GABA-Mediated Cl^- Influx. Topiramate, in common with some other AEDs, has been shown to enhance GABA-mediated Cl^- fluxes into neurons (48). Topiramate increased the frequency of GABA-mediated activation of $GABA_A$ receptors in cultures of cerebellar and cortical neurons (48). Ligand binding studies have demonstrated that TPM does not interact with the GABA or benzodiazepine binding sites on the $GABA_A$ receptor (49). Topiramate must therefore exert its effect on $GABA_A$-mediated currents through a novel interaction with the $GABA_A$ receptor, but the detailed mechanism still is not clear (48).

Glutamate Receptors. Topiramate has no effect on NMDA-type excitatory amino acid receptors, but inhibits the activity of KA on the AMPA/KA receptor subtype in a concentration-dependent manner (50,51). The blocking of KA-evoked potentials reduces neuronal excitability and could contribute to the drug's antiepileptic action.

Carbonic Anhydrase. Topiramate weakly inhibits the types II and IV isoenzymes of carbonic anhydrase (52). Intracellular bicarbonate ions have a depolarizing action when $GABA_A$ receptors are stimulated. This effect could be diminished by carbonic anhydrase inhibitors (54).

Topiramate and Neuroprotection. With the knowledge of the pharmacological properties of TPM, several of its mechanisms of action could be expected to have neuroprotective properties. By using the fluorescent probe calcein and immunocytochemistry, we were able to detect that significantly more neurons in primary culture treated with TPM survived during glutamate-induced excitotoxicity compared to neurons that had not been treated with TPM (Ängehagen et al, in preparation). Because it is well established that TPM has a negative modulatory effect on KA-induced Ca^{2+} transients and that overstimulation of AMPA/KA receptors induces cell death, we further investigated the hypothesis in which TPM is postulated to bind to the phosphorylation sites on the proteins it modulates. This working hypothesis developed from the fact that at the molecular level it was not known how TPM modulates the activity of AMPA/KA receptors. The hypothesis is based on earlier observations that okadaic acid inhibits the effect of TPM on AMPA/KA-receptor currents and that dibutyryl-cAMP promotes the restoration of AMPA/KA receptor currents after wash-out of TPM (50). This hypothesis has several predictive elements. One of them predicts that compounds that stimulate the activity of PKA should reduce the efficacy of TPM, whereas compounds that inhibit PKA should increase the relative activity of TPM. To be able to test this hypothesis we used the adenylyl cyclase stimulant forskolin (55) to raise the level of PKA phosphorylated receptors and increase AMPA/KA-induced currents. To block PKA from phosphorylating the channel complex and thereby reducing AMPA/KA-induced currents, we used the cAMP-dependent protein kinase inhibitor H89 (56). In short, the enhanced Ca^{2+} flux caused by forskolin was normalized after treatment with TPM, and the reduced Ca^{2+} flux was further reduced when TPM was added to the bath. Therefore, TPM affects PKA, but these experiments did not answer the question of whether the effect is on the phosphorylation site on the receptor channel, on PKA itself, or by manipulating the levels of cAMP. To investigate this we used the knowledge that TPM does not inhibit NMDA receptors and that the NMDA receptor is activated by PKA phosphorylation (57), results we also confirmed in our model system. Because TPM reduced KA-induced Ca^{2+} transients but not NMDA-induced transients, our findings indicate that TPM does not act directly on PKA itself, but support the concept that TPM probably binds selectively to phosphorylation sites on the AMPA/KA receptors (Fig. 3).

GENERAL DISCUSSION AND CONCLUSION

Gaining knowledge of basic mechanisms of action by which AEDs exert their antiepileptic effects at

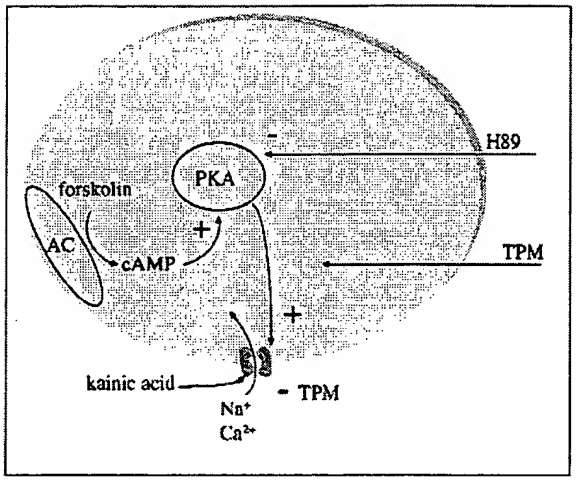

Fig. 3. Proposed action of topiramate (TPM) on kainate receptors. The drawing is a schematic representation of the proposed interaction between TPM and protein kinase A (PKA) mediated phosphorylation of kainate activated receptors. Forskolin was used to raise the levels of cAMP, thereby activate PKA and H89 to block PKA mediated phosphorylation. (AC, adenylate cyclase).

the cellular level is important for the development of modern CNS active drugs. AEDs may achieve their effects in a variety of ways: by altering membrane properties, by decreasing excitatory or increasing inhibitory action potentials at sites of seizure genesis or propagation, or by altering neuronal ionic environment. By reducing neuronal hyperexcitability during seizures it may be possible to reduce accumulation of Ca^{2+} ions in the cell, thereby preventing Ca^{2+}-induced cell death from occurring. Identification of the mechanisms involved in neuronal excitation makes it possible to create new, effective drugs against different acute and chronic pathological conditions in brain.

What is interesting about these three drugs is that by understanding the mechanism of action, new ideas about epilepsy and other neurological diseases can be forthcoming. Vigabatrin has been shown to have a selective and massive effect on brain GABA. While drugs that act only on the $GABA_A$ receptor as agonists are broad-spectrum AEDs such as phenobarbital and the benzodiazepines, a general increase in GABA is not generally antiepileptic and inhibitory. Indeed, this can be emphasized for the case of absence seizures. Vigabatrin and the expected increase in GABA can actually exacerbate this seizure type in both animals and humans (58–60). This observation has helped to lead to the understanding that epileptic seizures are not all the same and that there are some types that can arise from too much inhibition or hyperpolarization. Many of these seizure types are generated from the thalamus and are genetically determined (61). Tiagabine also has a broader GABA effect than simply as a receptor agonist influence, and again this is a narrow-spectrum drug not effective in primary generalized seizure (62).

On the other hand, TPM, with its GABA receptor agonist effect, Na^+ channel blocking effect, and especially the KA receptor blocking effect, is a broad-spectrum AED and also has effects on other neurological and systemic diseases. It is being tested as a prophylactic drug for migraine, as a mood stabilizer, and as an anti-diabetic drug. Over 70% of patients in clinical trials lose weight, and this is another fascinating result of this multiaction compound. Although vigabatrin has shown neuroprotective properties in animal models (63), TPM seems to have the most promise as a neuroprotectant (64), and this will surely be an exciting area that could be beneficial to patients with stroke or other diseases that can compromise the CNS and mental functioning.

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REFERENCES

1. Stefan, H., Halasz P., Gil-Nagel, A., Shorvon, S., Bauer, G., Ben Menachem, E., Perucca, E., Wieser, H. G., and Steinlein, O. 2001. Recent advances in the diagnosis and treatment of epilepsy. Eur. J. Neurol. 8:519–539.
2. Kwan, P. and Brodie, M. J. 2000. Early identification of refractory epilepsy. N. Engl. J. Med. 342:314–319.
3. Schwartz, R. D. 1988. The GABAA receptor-gated ion channel: Biochemical and pharmacological studies of structure and function. Biochem. Pharmacol. 37:3369–3375.
4. Salt, T. E., Eaton, S. A., and Turner, J. P. 1996. Characterization of the metabotropic glutamate receptors (mGluRs) which modulate GABA-mediated inhibition in the ventrobasal thalamus. Neurochem. Int. 29:317–322.
5. Hamberger, A. and van Gelder, N. M. 1993. Metabolic manipulation of neuronal tissue to counter the hypersynchronous excitation of migraine and epilepsy. Neurochem. Res. 18:503–509.
6. Greengard, P. 2001. The neurobiology of slow synaptic transmission. Science 294:1024–1030.
7. Traub, R. D., Jefferys, J. G., and Whittington, M. A. 1999. Functionally relevant and functionally disruptive (epileptic) synchronized oscillations in brain slices. Adv. Neurol. 79:709–724.
8. Ben-Ari, Y. and Cossart, R. 2000. Kainate, a double agent that generates seizures: Two decades of progress. Trends Neurosci. 23:580–587.
9. Lee, M C., Rho, J. L., Kim, M. K., Woo, Y. J., Kim, J. H., Nam, S. C., Suh, J. J., Chung, W. K., Moon, J. D., and Kim, H. I. 2001.

c-JUN expression and apoptotic cell death in kainate-induced temporal lobe epilepsy. J. Korean Med. Sci. 16:649–656.
10. Frantseva, M. V., Velazquez, J. L., Hwang, P. A., and Carlen, P. L. 2000. Free radical production correlates with cell death in an in vitro model of epilepsy. Eur. J. Neurosci. 12:1431–1439.
11. Sattler, R. and Tymianski, M. 2000. Molecular mechanisms of calcium-dependent excitotoxicity. J. Mol. Med. 78:3–13.
12. Mattson, M. P., Keller, J. N., and Begley, J. G. 1998. Evidence for synaptic apoptosis. Exp. Neurol. 153:35–48.
13. Sonnewald, U., Westergaard, N., and Schousboe, A. 1997. Glutamate transport and metabolism in astrocytes. Glia 21:56–63.
14. Reiter, R. J., Acuna-Castroviejo, D., Tan, D. X., and Burkhardt, S. 2001. Free radical-mediated molecular damage: Mechanisms for the protective actions of melatonin in the central nervous system. Ann. N. Y. Acad. Sci. 939:200–215.
15. Novelli, A., Reilly, J. A., Lysko, P. G., and Henneberry, R. C. 1988. Glutamate becomes neurotoxic via the *N*-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res. 451:205–212.
16. Sanchez-Carbente, M. R. and Massieu L. 1999. Transient inhibition of glutamate uptake in vivo induces neurodegeneration when energy metabolism is impaired. J. Neurochem. 72:129–138.
17. Dugan, L. L., Sensi, S. L., Canzoniero, L. M., Handran, S. D., Rothman, S. M., Lin, T. S., Goldberg, M. P., and Choi, D. W. 1995. Mitochondrial production of reactive oxygen species in cortical neurons following exposure to *N*-methyl-D-aspartate. J. Neurosci. 15:6377–6388.
18. Reynolds, I. J. and Hastings, T. G. 1995. Glutamate induces the production of reactive oxygen species in cultured forebrain neurons following NMDA receptor activation. J. Neurosci. 15:-3318–3327.
19. Perez Velazquez, J. L., Frantseva, M. V., and Carlen, P. L. 1997. In vitro ischemia promotes glutamate-mediated free radical generation and intracellular calcium accumulation in hippocampal pyramidal neurons. J. Neurosci. 17:9085–9094.
20. Butcher, S. P., Jacobson, I., and Hamberger, A. 1988. On the epileptogenic effects of kainic acid and dihydrokainic acid in the dentate gyrus of the rat. Neuropharmacology 27:375–381.
21. Elliot, K. A. C. and van Gelder, N. M. 1958. Occlusion and metabolism of gamma aminobutyric acid by brain tissue. J. Neurochem. 3:28–40.
22. Jung, M. F., Lippert, B., Metcalf, B. W., Bohlen, P., and Schechter, P. J. 1977. Gamma-vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: Effects on brain GABA metabolism in mice. J. Neurochem. 29: 797–802.
23. Gale, K. 1986. Role of the substantia nigra in GABA-mediated anticonvulsant actions. In: Delgado-Escueta, A. V., Ward, A. A., Woodbury, D. M., and Porter, R. J. (eds.). Basic Mechanisms of the Epilepsies: Molecular and Cellular Approaches. 44:343–364. Advances in Neurology.
24. Schechter, P. J., Tranier, Y., and Grove, J. 1979. Attempts to correlate alterations in brain GABA metabolism by GABA-T inhibitors with their anticonvulsant effects. Pages 43–57, in Mandel, P., DeFeudes, E. V. (eds.). GABA-Biochemistry and CNS Functions. New York, Plenum Press.
25. Schechter, P. J., Tranier, Y., Jung, M. J., and Bohlen, P. 1977. Audiogenic seizure protection by elevated brain GABA concentration in mice: effects of gamma-acetylenic gaba and gamma-vinyl GABA, two irreversible GABA-T inhibitors. Eur. J. Pharmacol. 45:319–328.
26. Meldrum, B. S. and Horton, R. 1978. Blockade of epileptic responses in the photosensitive baboon, Papio papio, by two irreversible inhibitors of GABA transaminase, gamma-acetylenic GABA (4-amino-hex-5-ynoic acid) and gamma-vinyl GABA (4-amino-hex-5-enoic acid). Psychopharmacology 59:47–50.
27. Grove, J., Schechter, P. J., Tell, G., Koch-Weser, J., Sjoerdsma, A., Warter, J. M., Marescaux, C., and Rumbach, L. Increased gamma-aminobutyric acid (GABA), homocarnosin and beta-alanine in cerebrospinal fluid of patients treated with gamma-vinyl GABA (4-amino-hex-5-enoic acid). Life Sci. 28:2431–2439.
28. Schechter, P. J., Hanke, N. F. J., Grove J., Huebert, N., and Sjoerdsma, A. 1984. Biochemical and clinical effects of gamma-vinyl GABA in patients with epilepsy. Neurology 134:34–39.
29. Pitkänen, A., Halonen, T., Ylinen, A., and Riekkinen, P. 1987. Somatostatin, β-endorphin, and prolactin levels in human cerebrospinal fluid during the gamma-vinyl GABA treatment of patients with complex partial seizures. Neuropeptides 9:185–195.
30. Sivenius, M. R. J., Ylinen, A., Murros, K., Matilainen, R., and Riekkinen, P. 1987. Double blind dose-reduction study of vigabatrin in complex partial epilepsy. Epilepsia 28:688–692.
31. Ben-Menachem, E., Persson, L. I., and Mumford, J. P. 1991. Effect of long term vigabatrin therapy on selected CSF neurotransmitter concentrations. J. Child. Neurology 6(Suppl 2):11–16.
32. Ben-Menachem, E., Persson, L., Schechter, P. J., Haegele, K. D., Huebert, N., Hardenberg, J., Dahlgren, L., and Mumford, J. P. 1988. Effects of single doses of vigabatrin on CSF concentrations of GABA, homocarnosine, homovanillic acid and 5-hydroxyindoleacedic acid in patients with complex partial seizures. Epilepsy Res. 2:96–101.
33. Ben-Menachem, E., Mumford, J., and Hamberger, A. 1993. Effect of long-term vigabatrin therapy on GABA and other amino acid concentrations in the central nervous system: A case study. Epilepsy Res. 16:241–243.
34. Ben-Menachem, E., Persson, L. I., Schechter, P. J., Haegele, K. D., Huebert, N., Hardenberg, J., Dahlgren, L., and Mumford, J. P. 1989. The effect of different vigabatrin treatment regimens on CSF biochemistry and seizure control in epileptic patients. Br. J. Clin. Pharmac. 27:79S–85S.
35. Kälviäinen, R., Halonen, T., Pitkänen, A., and Riekkinen, P. J. 1993. Amino acid levels in the cerebrospinal fluid of newly diagnosed epileptic patients: Effect of vigabatrin and carbamazepine monotherapy. J. Neurochem. 60:1244–1250.
36. Petroff, O. A. C., Rothman, D. L., Behar, K. L., and Mattson, R. H. 1995. Initial observations of effect of vigabatrin on in vivo ^{1}H spectroscopic measurements of γ-aminobutyric acid, glutamate, and glutamine in human brain. Epilepsia 36:457–464.
37. Spanaki, M. V., Siegel, H., Kopylev, L., Fazilat, S., Dean, A., Liow, K., Ben-Menachem, E., Gaillard, W. D., and Theodore, W. H. 1999. The effect of vigabatrin (gamma-vinyl GABA) on cerebral blood flow and metabolism. Neurology 53:1518–1522.
38. Richens, A. 1991. Pharmacology and clinical pharmacology of vigabatrin. J. Child. Neurology 6:2S7–2S10.
39. Dalby, N. O. 2000. GABA-level increasing and anticonvulsant effects of three different GABA uptake inhibitors. Neuropharmacology 39:2399–2407.
40. Sills, G. J., Patsalos, P. N., Butler, E., Forrest, G., Ratnaraj, N., and Brodie, M. J. 2001. Visual field constriction: Accumulation of vigabatrin but not tiagabine in the retina. Neurology 57:196–200.
41. Kalviainen, R. 2001. Long-term safety of tiagabine. Epilepsia 42(Suppl 3):46–48.
42. Marson, A. G., Kadir, Z. A., Hutton, J. L., and Chadwick, D. W. 1997. The new antiepileptic drugs: A systematic review of their efficacy and tolerability. Epilepsia 38:859–880.
43. Shank, R. P., Gardocki, J. F., Vaught, J. L., Davis, C. B., Schupsky, J. J., Raffa, R. B., Dodgson, S. J., Nortey, S. O., and Maryanoff, B. E. 1994. Topiramate: preclinical evaluation of structurally novel anticonvulsant. Epilepsia 35:450–460.
44. Montouris, G. D., Biton, V., and Rosenfeld, W. E. 2000. Non-focal generalized tonic-clonic seizures: response during long-term topiramate treatment. Topiramate YTC/YTCE Study Group. Epilepsia 41(Suppl 1):S77–S81.
45. Sachdeo, R. C., Glauser, T. A., Ritter, F., Reife, R., Lim, P., and Pledger, G. 1999. A double-blind, randomized trial of topiramate in Lennox-Gastaut syndrome. Topiramate YL Study Group. Neurology 52:1882–1887.
46. Perucca, E. 2001. Clinical pharmacology and therapeutic use of the new antiepileptic drugs. Fundam Clin. Pharmacol. 15:405–417.

47. Zona, C., Ciotti, M. T., and Avoli, M. 1997. Topiramate attenuates voltage-gated sodium currents in rat cerebellar granule cells. Neurosci. Lett. 231:123–126.
48. White, H. S., Brown, S. D., Woodhead, J. H., Skeen, G. A., and Wolf, H. H. 1997. Topiramate enhances GABA-mediated chloride flux and GABA-evoked chloride currents in murine brain neurons and increases seizure threshold. Epilepsy Res. 28:167–179.
49. Gordey, M., DeLorey, T. M., and Olsen, R. W. 2000. Differential sensitivity of recombinant GABA(A) receptors expressed in Xenopus oocytes to modulation by topiramate. Epilepsia 41 (Suppl 1):S25–S29.
50. Gibbs, J. W. III., Sombati, S., DeLorenzo, R. J., and Coulter, D. A. 2000. Cellular actions of topiramate: Blockade of kainate-evoked inward currents in cultured hippocampal neurons. Epilepsia 41(Suppl. 1):S10–S16.
51. Ängehagen, M., Shank, R., Hansson, E., Rönnbäck, L., and Ben-Menachem, E. 2001. Topiramate affects the ability of protein kinase to phosphorylate glutamate receptors activated by kainate. Epilepsia 42(Suppl. 7):S10.
52. Dodgson. S. J., Shank, R. P., and Maryanoff, B. E. 2000. Topiramate as an inhibitor of carbonic anhydrase isoenzymes. Epilepsia 41(Suppl. 1):S35–S39.
53. White, H. S. 1999. Comparative anticonvulsant and mechanistic profile of the established and newer antiepileptic drugs. Epilepsia 40(Suppl. 5):S2–S10.
54. Herrero, A. I., Del Olmo, N., Gonzales-Escalada, J. R. and Solis, J. M. 2002. Two new actions of topiramate: Inhibition of depolarizing GABA(A)-mediated responses and activation of a potassium conductance. Neuropharmacology 42:210–220.
55. Huang, R. D., Smith, M. F., and Zahler, W. L. 1982. Inhibition of forskolin-activated adenylate cyclase by ethanol and other solvents. J. Cyclic Nucleotide Res. 8:385–394.
56. Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshioka, T., and Hidaka, H. 1990. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. J. Biol. Chem. 265:5267–5272.
57. Raman, I. M., Tong, G., and Jahr, C. E. 1996. Beta-adrenergic regulation of synaptic NMDA receptors by cAMP-dependent protein kinase. Neuron 16:415–421.
58. Aker, R. G., Ozkara, C., Dervent, A., and Yilmaz Onat, F. 2002. Enhancement of spike and wave discharges by microinjection of bicuculline into the reticular nucleus of rats with absence epilepsy. Neurosci. Lett. 322:71–74.
59. Panayiotopoulos, C. P. 1999. Importance of specifying the type of epilepsy. Lancet 354:2002–2003.
60. Panayiotopoulos, C. P., Agathonikou, A., Sharoqi, I. A., and Parker, A. P. 1997. Vigabatrin aggravates absences and absence status. Neurology 149:1467.
61. Slaght, S. J., Leresche, N., Deniau, J. M., Crunelli, V., and Charpier, S. 2000. Activity of thalamic reticular neurons during spontaneous genetically determined spike and wave discharges. J. Neurosci. 22:2323–2334.
62. Knake, S., Hamer, H. M., Schomburg, U., Oertel, W. H., and Rosenow, F. 1999. Tiagabine-induced absence status in idiopathic generalized epilepsy. Seizure 8:314–317.
63. Andre, V., Ferrandon, A., Marescaux, C., Nehlig, A. 2001. Vigabatrin protects against hippocampal damage but is not antiepileptogenic in the lithium-pilocarpine model of temporal lobe epilepsy. Epilepsy Res. 47:99–117.
64. Yang, Y., Li, Q., and Shuaib, A. 2000. Enhanced neuroprotection and reduced hemorrhagic incidence in focal cerebral ischemia of rat by low dose combination therapy of urokinase and topiramate. Neuropharmacology 39:881–888.

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Novel dopamine releasing response of an anti-convulsant agent with possible anti-Parkinson's activity

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Summary. We used cerebral microdialysis to assess the ability of the anti-convulsant drug Zonisamide (ZNS) to release striatal dopamine in 6-hydroxydopamine nigrotomized rats. Following exogeneously administered ZNS we measured dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels in striatal dialysates obtained from the ipsilateral side of the nigrotomy. ZNS administration alone had no effect on levels of DA and its metabolites or rotational behavior. Administration of carbidopa-levodopa alone led to small but insignificant increases in rotational behavior contralateral to the side of the nigrotomy but no corresponding increases in indices of striatal catecholamine release. In contrast, if animals were preloaded with carbidopa and ZNS was co-administered with levodopa 30 minutes later significant increases in contralateral rotational behavior occurred within 20 minutes of ZNS-levodopa injection that lasted for at least 90 minutes. In contrast to the uniform rotational behavioral responses observed in all our nigrotomized animals, less than half demonstrated neurochemical evidence of DA release. In these "responder" animals DOPAC levels increased 300% following carbidopa-levodopa-ZNS administration. We conclude that these results support previously reported findings and provide additional evidence that the anticonvulsant ZNS appears to possess anti-Parkinson's properties. ZNS could therefore be a novel agent for the treatment of PD that could delay the use of or reduce the amount of levodopa needed to treat patients with PD.

Keywords: Parkinson's disease, Zonisamide, dopamine, 6-hydroxydopamine, microdialysis, behavior.

Introduction

Parkinson's disease (PD) is the most common neurodegenerative movement disorder affecting older adults. Neuropathologically, the disease is character-

ized by a relative selective loss of dopaminergic projection neurons within the substantia nigra pars compacta (SNc), with formation of cytoplasmic inclusions (Lewy bodies) within many surviving neurons (Hornykiewicz, 1963; Bethlem and Den Hartog Jager, 1960). Reductions of SNc dopamine (DA) neurons leading to greater than 80% depletion of striatal dopamine (Bernheimer et al., 1973) results in the development of the classical signs of PD (Hoehn and Yahr, 1967; Fahn et al., 1971; DiRocco et al., 1996).

The most efficacious treatment for PD is replacement of DA by orally administered levodopa. In contrast to DA levodopa readily crosses the blood-brain barrier, is taken up by DA neurons via the aromatic amino acid transporter and is converted to DA by the action of amino acid (L-dopa) decarboxylase (Birkmeyer, 1969; Sourkes, 1972; Celesia and Wanamaker, 1976; de Belleroche and Bradford, 1976). Administration of levodopa however is frequently associated with a variety of adverse side effects including somnolence, nausea, hallucinations and hypotension (Yahr et al., 1968, 1969). Furthermore, many patients develop tolerance to the drug leading to escalating doses and in a large majority of patients chronic use of high levodopa dosages results in the development of abnormal involuntary dyskinetic movements (Jankovic, 2000; Martignoni et al., 2003). Consequently, in many patients at optimal doses or with long-term levodopa administration dyskinesias or adverse side effects frequently develop to the point where drug doses are decreased (Melamed et al., 1999; van Laar, 2003; Olanow, 2003).

Investigators have sought to identify pharmacologic agents capable of delaying the administration of levodopa and hence obviate the many side effects induced by the drug. One such approach has been to identify drugs that could increase striatal dopamine release from surviving nigro-striatal DA nerve terminals. Powerful dopamine releasing and depleting agents such as reserpine or tetrabenazine lead to long-term sustained reductions of dopamine leading to exacerbation of PD symptoms (Fekete et al., 1980; Fahn, 1983; Tolwani et al., 1999). Therefore, an ideal therapeutic agent in principle would be one that increases vesicular dopamine release without depleting DA vesicle storage completely.

In a previous in-vivo microdialysis study (Okada et al., 1995) the authors reported that the anticonvulsant Zonisamide (ZNS) had potential anti-parkinsonian activities within the striatum since the drug was able to modestly increase extracellular striatal levels of DA and its metabolites following a single injection in normal male rats. Furthermore, three weeks of daily ZNS administration led to increases in intracellular levels of striatal DA, suggesting that ZNS may have the novel properties of increasing DA storage and potentiating vesicle release within DA nerve terminals innervating the striatum. Subsequently, Murata and colleagues carried out a small preliminary 12-week open-labeled clinical drug trial with nine PD patients receiving ZNS and reported improvement of parkinsonian symptoms in the majority of patients (Murata et al., 2001). In a follow-up report the same investigators observed beneficial longer-term effects of 1–2 years in approximately half of their patients taking ZNS (Murata et al., 2003). Taken together, these findings suggest therapeutic benefit for ZNS in the treatment of PD. These findings were

particularly important since there is a definite need for adjuvant drugs that have value in the treatment of PD which avoid the adverse side-effects of levodopa itself.

In the present study we examined the ability of ZNS to increase striatal dopamine release in 6-hydroxydopamine (6-OHDA) lesioned rats to determine whether similar pharmacologic properties were observed in a rodent model of PD. Following an intraperitoneal (i.p.) injection of ZNS alone or in combination with levodopa/carbidopa levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in the striatum on the ipsilateral side of the lesion using in-vivo cerebral microdialysis techniques.

The results of the present study show that all of the 6-OHDA animals treated with a combination of levodopa-carbidopa and ZNS showed significant 300%–400% increases in levodopa-induced contralateral rotational behavior compared with ZNS or levodopa-carbidopa treatment alone. In contrast, less than half of the animals showed significant increases in striatal DA release (as evidenced by increased DOPAC levels) in association with the increased rotational behavior. These findings provide strong pharmacologic evidence for striatal DA releasing effects by ZNS in a rodent model of PD although neurochemical indices sometimes failed to show associated evidence for catecholamine release. This preliminary study therefore suggests that ZNS may be a novel releaser of striatal DA in Parkinson's patients treated with levodopa.

Materials and methods

D-methamphetamine, DA, DOPAC, HVA, carbidopa, L-dopa methyl ester and (–) apomorphine were purchased from Sigma/Aldrich (St. Louis, MO). All other reagent grade chemicals and organic solvents used in the study were of the highest-grade purity available. ZNS (base form) was obtained as a gift from Dainippon Pharmaceutical Co., Ltd, Tokyo, Japan.

All in-vivo experiments were conducted in Sprague-Dawley male rats (14–20 weeks) from Charles River Laboratories (Wilmington, MA, USA) in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the local Animal Care Committee. Rats were housed individually at 20–22°C on a 12-h light-dark cycle with food and water available ad libitum.

Efficacy of 6-OHDA nigrotomy lesions (left substantia nigra) was assessed by rotational behavior provoked by apomorphine administration. Commercially available 6-OHDA lesioned rats (Charles River Laboratories) that received the lesioning procedure 1–2 months earlier were pre-selected based on measurement of their circling activity. Studies of circling behavior based on the paradigm developed by Ungerstedt et al. (1971) were performed on the lesioned rats. All rats were tested with (–) apomorphine 0.2 mg/kg, subcutaneous and only those circling briskly (>20 turns/5 min) contralaterally to the side of the nigrotomy were used in the study: Rotational rates (per 5 minutes): Animal #1 (63) Animal #2 (91) Animal #3 (57) Animal #4 (63) Animal #5 (61).

Microdialysis guide cannulae (CMA/microdialysis AB, Sweden) were implanted under deep ketamine/xylaxine anesthesia in the striatum in a stereotaxic frame (from Bregma: +0.6 AP; +3.0 L; −4.7 V) ipsilateral to the nigrotomy. After 10–12 days following cannulae placement, a microdialysis probe 2 mm membrane length and 20 kD cut-off (CMA 12/microdialysis AB Sweden) was inserted into the rats guides. The probes were perfused at 1 ul/min with buffered (pH 7.2) artificial cerebrospinal fluid composed of 148 mM NaCl, 3 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 0.866 mM Na_2HPO_4, 0.195 mM $NaHPO_4$. Probe recovery efficiencies averaged 15%.

For microdialysis studies acute release of DA and its metabolites following probe insertion resolved within 100 min after which stable dialysate levels were reached. Animals were fasted for two hours prior to dialysis and provided only water during the experiment. All four experiments

described were carried out in each of the five animals, separated by 7–10 days following completion of each experiment. The first experiment was carried out 10–12 days following cannulae placement surgery. In all experiments levodopa was administered as levodopa-methyl-ester. *Experiment #1*: ZNS alone; after collection of baseline dialysate samples every 25 min for 100 min animals were given aqueous sodium ZNS (40 mg/kg i.p. × 1) and samples were collected for an additional 100 min. *Experiment #2*: Carbidopa/levodopa; animals were given carbidopa (25 mg/kg i.p.) 100 minutes after baseline stabilization. 30 minutes after carbidopa treatment the animals were administered levodopa-methyl ester (5 mg/kg i.p.) and dialysates were collected for an additional 100 min. *Experiment #3*: Carbidopa/levodopa/ZNS; animals were given ZNS (40 mg/kg i.p) 100 min after baseline stabilization. 75 minutes after ZNS injection carbidopa (25 mg/kg i.p.) was given. 30 minutes later animals were co-administered levodopa-methyl ester (5 mg/kg i.p.) and ZNS (40 mg/kg i.p) together as a single injection and dialysates were collected for an additional 100 min. *Experiment #4*: Carbidopa/levodopa/methamphetamine; animals were given carbidopa (25 mg/kg i.p.) 100 minutes after baseline stabilization. 30 minutes after carbidopa treatment the animals were co-administered levodopa-methyl ester (5 mg/kg i.p.) and D-methamphetamine (10 mg/kg i.p) together as a single injection and dialysates were collected for an additional 100 min. In these methamphetamine experiments 2 of 5 animals expired before the second dialysis sample was obtained and these data were omitted. Rotational behavioral responses were recorded during the course of the experiment.

Striatal dialysates were collected into a refrigerated (0°C–4°C) fraction collector and analyzed immediately. The concentrations of DA, HVA and DOPAC were measured in the microdialysis samples by an "on line" system of high-performance liquid chromatography and ESA Coulochem II electrochemical detection (HPLC-ED). The HPLC-ED system used a Brownlee RP-18 5 micron narrow bore cartridge (2.1 mm ID × 3 cm L), a 20 ul injection loop, a ESA electrochemical detector and cell (Bioanalytical Systems) and a 746 Waters Data Module integrator. One liter of mobile phase at final pH 3.10 contained 7% v/v acetonitrile, 6.5% v/v tetrahydrofuran, 1.1% v/v diethylamine, 10.0 mM NaCl, 32.2 mM sodium citrate, 14.5 mM sodium phosphate monobasic, 0.025 mM EDTA, and 1.94 mM 1-octane sulfonic acid and flow rates were 1 ml/min. For each experiment carried out fresh aqueous standards were injected (100–200 pg/20 μL injection) to the HPLC-ED system to confirm retention time and peak height of the neurochemicals.

Following completion of the final experiments rats were sacrificed by rapid decapitation. Brains were immediately removed and immersed into 4% paraformaldehyde overnight, then into 30% sucrose until sinking. 50 micron sections were stained with cresyl violet to verify probe placement within the striatum.

Group differences between mean striatal DA, DOPAC and HVA levels following treatment with ZNS, carbidopa-levodopa or in combination were analyzed by one-way analysis of variance (ANOVA) and Newman-Keuls testing. Student's t-test was applied for individual animals in the analysis of the differences between the effects of the different dosing paradigms on release of extracellular DA, DOPAC and HVA levels.

Results

Effects on rotational behavior following administration of ZNS, carbidopa-levodopa or methamphetamine (Fig. 1)

Rotational behaviors were observed in all animals examined and were contralateral to the side of the nigrotomy. No rotational responses were observed following ZNS treatment alone and a mild non-significant increase to a rate of 5 rotations per hour which failed to reach significance ($p > 0.05$, one-way ANOVA with Newman-Keuls Multiple Comparison Test) was found with carbidopa-levodopa treatment alone. There were highly significant increases in rotation rates (range 15–40 per hour) when ZNS was co-administered with levodopa-carbidopa ($p < 0.01$, levodopa-carbidopa vs. levodopa-carbidopa-

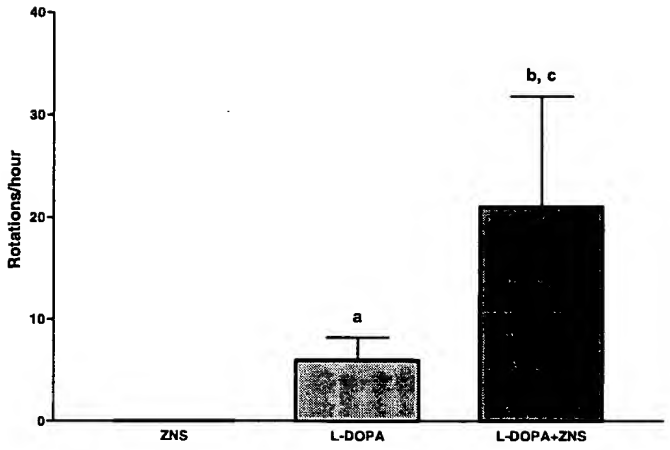


Fig. 1. Circling activity of 6-OHDA lesioned rats following administration of ZNS alone, carbidopa-levodopa or a combination of ZNS-carbidopa-levodopa, as described in Materials and Methods. Circling was contralateral to the side of the nigrotomy. The data are expressed as mean + SD, N = 5. Comparison between different treatment arms demonstrates a significant increase in rotational behavior following ZNS-carbidopa-levodopa treatment. [(a) $p > 0.05$, ZNS vs. levodopa; (b) $p < 0.01$, levodopa-carbidopa vs. ZNS-levodopa-carbidopa; (c) $P < 0.001$, ZNS versus ZNS-levodopa-carbidopa]

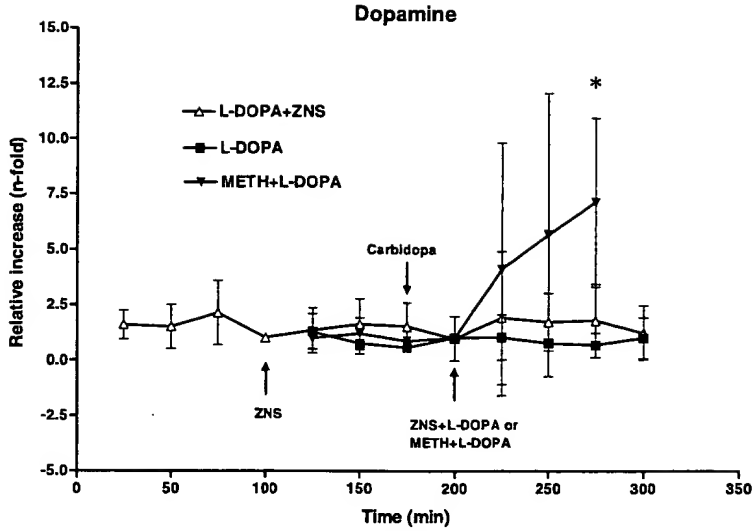
ZNS; $p < 0.001$, ZNS vs. levodopa-carbidopa-ZNS). In a separate analysis, as a comparative standard, methamphetamine-carbidopa-levodopa was administered and was found to increase rotation rates 3-fold compared to rates observed following levodopa-carbidopa-ZNS treatment (Fig. 3).

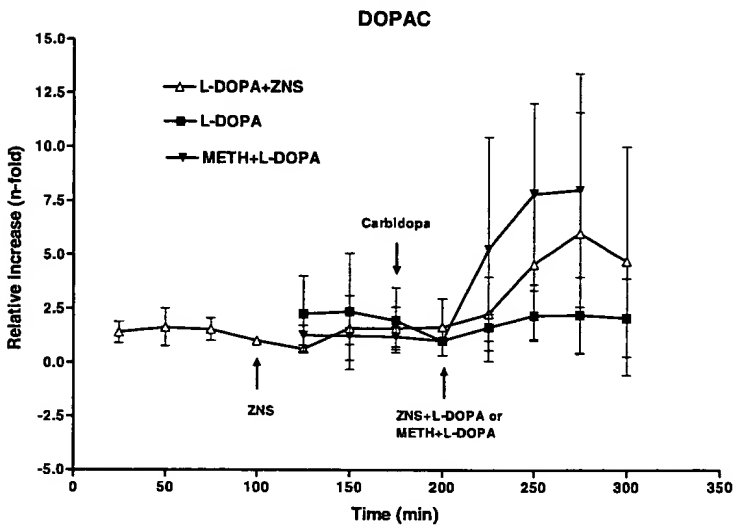
Group effects on striatal DA, DOPAC and HVA release following administration of ZNS with and without carbidopa/levodopa loading (Fig. 2)

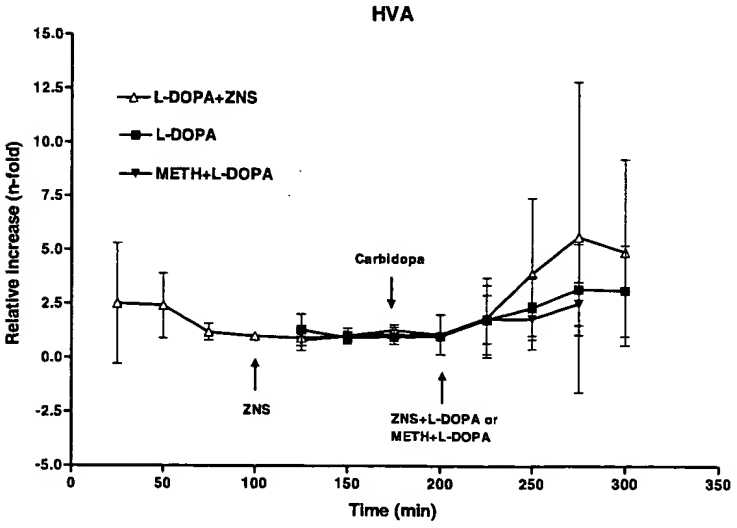
Extracellular striatal DA, DOPAC and HVA levels following ZNS administration to 6-OHDA-treated animals were measured and are expressed as n-fold changes relative to stabilized controls. The basal levels (average + SD) of DA, DOPAC and HVA were 27.0 + 26.0, 30.0 + 16.0 and 23.4 + 22.0 pg/20 μL injection, respectively. Following baseline stabilization, ZNS administration did not significantly increase release of DA, DOPAC or HVA ($Ps > 0.05$) 100 min after treatment. Similar results were found when animals were administered levodopa-carbidopa alone and an insignificant trend towards increased DOPAC release was observed when levodopa-carbidopa-ZNS was administered in combination.

Effects on striatal DA, DOPAC and HVA release in individual animals following administration of ZNS with and without carbidopa/levodopa loading (Fig. 3)

Because of the heterogeneity of neurochemical responses to ZNS and carbidopa-levodopa leading to large variances in the group data a sub-analysis of the responses in individual animals was performed. Two of five animals [animal


Dopamine
L-DOPA+ZNS
L-DOPA
METH+L-DOPA
Carbidopa
ZNS
ZNS+L-DOPA or
METH+L-DOPA
*
Relative Increase (n-fold)
Time (min)


DOPAC
L-DOPA+ZNS
L-DOPA
METH+L-DOPA
Carbidopa
ZNS
ZNS+L-DOPA or
METH+L-DOPA
Relative Increase (n-fold)
Time (min)


HVA
L-DOPA+ZNS
L-DOPA
METH+L-DOPA
Carbidopa
ZNS
ZNS+L-DOPA or
METH+L-DOPA
Relative Increase (n-fold)
Time (min)

(57) and animal (60)] showed statistically significant increases in DOPAC elevations when ZNS was co-administered with carbidopa-levodopa compared to ZNS or carbidopa-levodopa alone (Ps = 0.01–0.02, Student's t-test), although no significant changes were observed in DA or HVA levels (data not shown). Animal (61) showed a trend towards increased DOPAC release and the remaining two animals showed no change in DOPAC, DA or HVA levels.

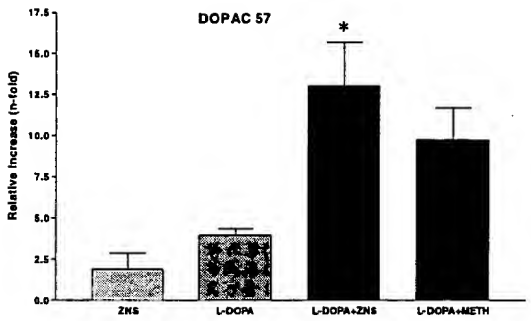
Effects on striatal DA release following administration of methamphetamine and carbidopa/levodopa loading

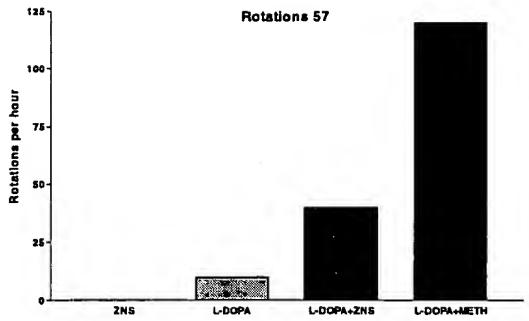
Neurochemical measurements were made with a more potent DA releaser, d-methamphetamine (with carbidopa-levodopa), to assess the relative DA releasing potency of ZNS-carbidopa-levodopa combination. Using a methamphetamine dose of 10 mg/kg, at maximal effect methamphetamine co-administered with carbidopa-levodopa led to significant 4–5-fold increases in striatal DA ($p = 0.018$, $N = 3$) but not HVA levels 75 minutes after treatment compared to ZNS-carbidopa-levodopa (Fig. 2). In contrast, DOPAC levels induced by methamphetamine-levodopa and ZNS-levodopa were not significantly different.

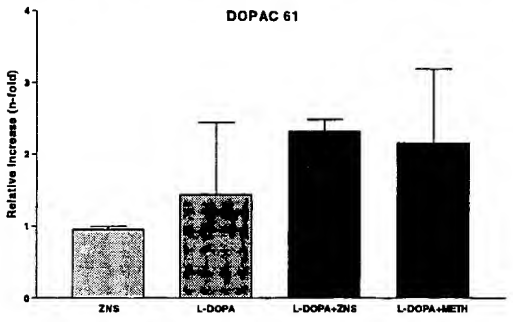
Discussion

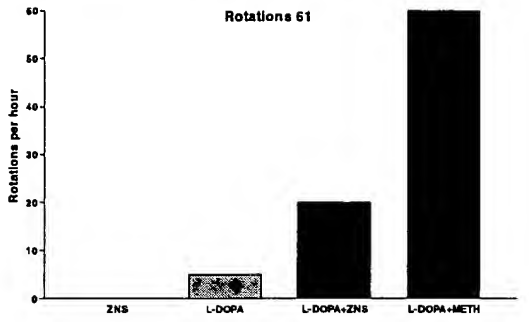
Levodopa remains the most efficacious drug in the treatment of PD despite the availability of other alternate dopamine agonists. Drug therapy is complicated however by the development of adverse side effects and long-term treatment leads to fluctuations in the response to levodopa resulting in complications such as "on-off phenomenon" and abnormal involuntary dyskinetic movements (De Jong et al., 1987; Nutt, 1990; Nutt et al., 1992). The development of motor complications is most strongly associated with escalating doses of levodopa (Koller, 1996; Stocchi et al., 1997). In attempts to reduce the dosage of levodopa while maintaining its overall efficacy inhibitors of monoamine oxidase-B and catechol-O-methyltransferase can be concurrently administered to lengthen the half-lives of levodopa by reducing its metabolism to DOPAC and HVA. However, the search for pharmacologic agents capable of increasing dopamine synthesis and release by surviving nerve terminals has been met with very limited success.

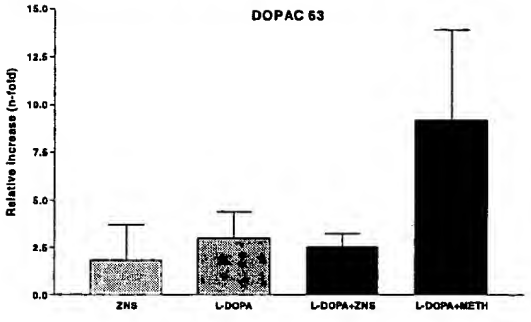
Fig. 2. Effects of ZNS on extracellular DA, DOPAC and HVA levels in striatal perfusates. Ordinate indicates relative n-fold increases in levels of DA, DOPAC and HVA (mean + SD, $N = 5$) and abscissa shows time in minutes. Effects by zonisamide (40 mg/kg) alone are shown (open triangles) between minutes 100–200. In these same experiments animals were subsequently administered carbidopa (25 mg/kg) at 175 minutes and ZNS (40 mg/kg) & levodopa (4 mg/kg) 30 minutes later. Effects of carbidopa-levodopa (closed squares) or methamphetamine-levodopa (closed triangles) given alone are shown; carbidopa was administered 30 min before levodopa or methamphetamine (10 mg/kg) & levodopa. Zonisamide alone showed no effects on DA, DOPAC or HVA levels. In combination with levodopa ZNS showed non-significant trends towards increased levels of DOPAC and HVA (Ps > 0.05). At maximal effect methamphetamine co-administered with carbidopa-levodopa led to significant 4-fold increases in striatal DA (*: $P = 0.018$) but not HVA or DOPAC levels 75 minutes after treatment compared to ZNS-carbidopa-levodopa

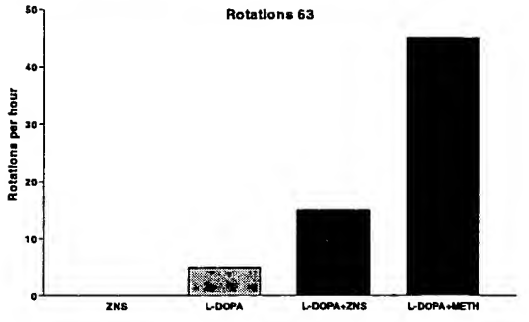

DOPAC 57
*
Relative Increase (n-fold)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
ZNS
L-DOPA
L-DOPA+ZNS
L-DOPA+METH

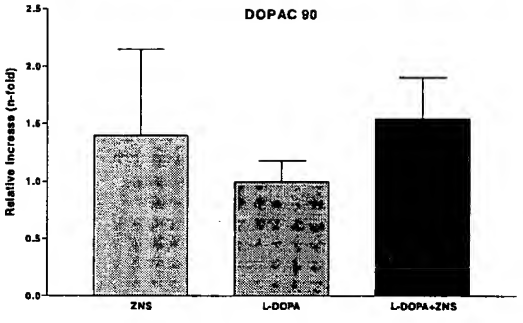

Rotations 57
Rotations per hour
125
100
75
50
25
0
ZNS
L-DOPA
L-DOPA+ZNS
L-DOPA+METH

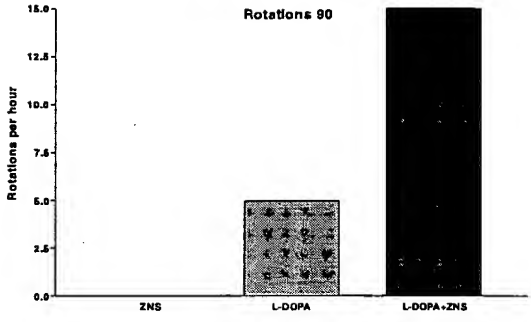

DOPAC 61
Relative Increase (n-fold)
4
3
2
1
0
ZNS
L-DOPA
L-DOPA+ZNS
L-DOPA+METH

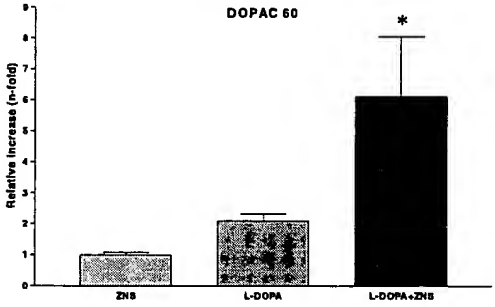

Rotations 61
Rotations per hour
60
50
40
30
20
10
0
ZNS
L-DOPA
L-DOPA+ZNS
L-DOPA+METH

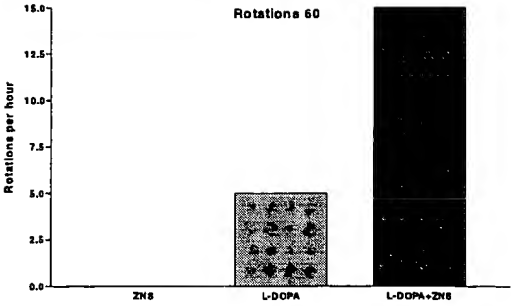

DOPAC 63
Relative Increase (n-fold)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
ZNS
L-DOPA
L-DOPA+ZNS
L-DOPA+METH


Rotations 63
Rotations per hour
50
40
30
20
10
0
ZNS
L-DOPA
L-DOPA+ZNS
L-DOPA+METH


DOPAC 90
Relative Increase (n-fold)
2.5
2.0
1.5
1.0
0.5
0.0
ZNS
L-DOPA
L-DOPA+ZNS


Rotations 90
Rotations per hour
15.0
12.5
10.0
7.5
5.0
2.5
0.0
ZNS
L-DOPA
L-DOPA+ZNS


DOPAC 60
*
Relative Increase (n-fold)
8
7
6
5
4
3
2
1
0
ZNS
L-DOPA
L-DOPA+ZNS


Rotations 60
Rotations per hour
15.0
12.5
10.0
7.5
5.0
2.5
0.0
ZNS
L-DOPA
L-DOPA+ZNS

Drugs such as amantidine and amitryptiline have demonstrated modest nerve terminal levodopa releasing properties in laboratory studies but clinical studies have not shown clear efficacy in facilitating reduction of levodopa dosages (Von Voigtlander and Moore, 1971; Ghosh and Hrdina, 1977). ZNS, an anticonvulsant recently approved for use in the United States, has been investigated as an anti-Parkinsonism agent based on animal studies demonstrating an ability of the drug to increase striatal dopamine release (Okada et al., 1995).

An in-vivo microdialysis study carried out by Okada et al. demonstrated that striatal dopamine release in normal adult rats was increased 15–30 minutes after a single 50 mg/kg i.p. injection of ZNS. Levels of DA and HVA but not DOPAC were increased about 15% at maximal effects and the elevations persisted for 60–90 minutes. In the same study the investigators further reported that ZNS, 50 mg/kg given orally daily for three weeks, produced increased intracellular striatal levels of dopamine, DOPAC and HVA. Subsequently, results from preliminary clinical drug trials suggested therapeutic benefit by ZNS in alleviating Parkinsonian symptoms (Murata et al., 2001, 2003). Taken together these findings suggested that ZNS could be a possible novel agent that may have value in the treatment of PD particularly in regard to avoiding the adverse side effects of levodopa itself.

In the present study, using 6-OHDA lesioned rats, we assessed whether ZNS could acutely increase striatal DA release in an animal model of PD. All animals demonstrated nearly identical increases in levodopa-provoked rotational behavior when ZNS was co-administered with carbidopa-levodopa. The increased rotational behavior induced by ZNS-levodopa appears to demonstrate clear pharmacologic efficacy in the ability of ZNS to release DA into the striatum. Secondly, contralateral rotational behavior demonstrates release of DA from the nigrotomized striatum indicating that ZNS is acting presumptively by releasing DA from surviving nerve terminals onto hypersensitive postsynaptic striatal DA receptors. It should be noted also that the rotation rates obtained with ZNS-levodopa-carbidopa were one-third of that obtained with high doses of methamphetamine-carbidopa-levodopa, suggesting that ZNS has the potential for inducing a relatively robust pharmacologic response. These behavioral studies therefore suggest that ZNS may indeed be an anticonvulsant with novel anti-Parkinson's properties and that it could be a novel agent for the treatment of PD that could delay the use of levodopa or reduce the amount of levodopa needed to treat persons with PD.

Fig. 3. Sub-group analysis of the effects of ZNS on extracellular DOPAC levels in striatal perfusates compared with rotational behavioral responses, in individual experimental animals. Mean levels of DOPAC for each individual animal are expressed as relative n-fold increases (mean + SD, N = 4 points) from baseline levels and were calculated by taking the average DOPAC values obtained from samples taken every 25 min over the entire 100 min time-course. All animals showed similar increases in rotational behavior (right-sided panels) when ZNS was co-administered with levodopa. In contrast, only 2 of 5 animals (top and bottom left panels) showed statistically significant increases in DOPAC levels following ZNS-carbidopa-levodopa administration compared to ZNS or levodopa-carbidopa treatment alone (*: Ps = 0.01–0.02). These data suggest that following combination ZNS/levodopa administration a pharmacological behavioral response can occur without a corresponding focal striatal neurochemical response

In contrast to strong pharmacologic evidence for increased striatal DA release by ZNS and in comparison to the findings of Okada et al. in normal rats, we found no demonstrable increases in striatal DA or its metabolites up to 2 hours after a single dose of ZNS (40 mg/kg) alone and no provocative rotational behavioral was observed. The lack of DA release from the ipsilateral side of the 6-OHDA treated animals following ZNS administration may simply be accounted for by the relative lack of DA within surviving terminals on the lesioned side itself or alternatively that 6-OHDA animals may require higher doses to induce release of DA. Nevertheless, since measurements of DA and its metabolites were not obtained from the contralateral striatum it is possible that ZNS may have increased release of DA on the contralateral side of the lesion. However, as 6-OHDA lesioned animals are sensitive behaviorally to DA release within the striatum (Hefti et al., 1980; Brannan et al., 1998) and no rotational behavior was observed ipsilateral to the nigrotomy following ZNS administration it is likely that if DA release from the contralateral striatum occurred the increases were not pharmacologically significant.

To determine whether levodopa loading could increase the efficacy of ZNS we carried out parallel microdialysis experiments to determine whether ZNS-levodopa combination would increase striatal DA levels in 6-OHDA animals pre-treated with carbidopa. In contrast to ZNS given alone, pre-treatment with carbidopa followed by co-administration of ZNS and levodopa 30 minutes later led to significant increases of DOPAC levels in a several of the animals which lasted 75 minutes. In these "responder" animals DOPAC was increased 300% versus levels obtained with levodopa/carbidopa treatment alone, indicating that ZNS induced a robust neurochemical response indicative of DA release. These results however are in contradiction with those reported by Okada who observed only 10%–20% increases in striatal DA and HVA with no effect on DOPAC levels in normal rats treated with equivalent doses of ZNS. The present design of the experiments limits our ability to directly compare data between normal and 6-OHDA lesioned animals and therefore it is unclear as to why the findings with 6-OHDA animals are very different from controls.

The precise reasons for the dissociation between neurochemical and pharmacologic responses in the remaining animals are also unclear. It is possible that surviving DA nerve terminal populations within the striatum on the ipsilateral side of the lesion may be distributed in a heterogeneous pattern such that placement of the dialysis catheter may hit areas of significant de-innervation. Such a condition would still maintain the animal's ability to manifest a pharmacologic behavioral response because of the recruitment of diffuse innervated regions remaining within the striatum. On the other hand, when these "non-responders" were treated with methamphetamine-carbidopa-levodopa, DA and DOPAC levels were increased suggesting that nerve terminals were responsive to pharmacologic stimulation. It is therefore possible that instrumental or experimental error may account for the discrepancies with the neurochemical non-responder animals that respond pharmacologically.

In summary, our preliminary study findings suggest that all animals treated with ZNS-carbidopa-levodopa demonstrated strong behavioral evidence for striatal DA release. In contrast, less than half of the 6-OHDA lesioned animals

studied showed neurochemical evidence for significant elevated DA release (as measured by increased DOPAC levels). Responses were observed 25 minutes following co-administration of ZNS and levodopa and persisted for at least one hour.

Finally, mechanisms underlying anti-convulsant properties are not fully understood and even less is known about the biochemical correlations between anti-convulsant properties and their effects on catecholamine release. It is therefore unusual to come across an agent such as ZNS that has both characteristics. Future investigations elucidating the mechanisms underlying catecholamine release by ZNS may potentially open up new vistas for discovering new classes of drugs that can be used as levodopa sparing agents in the treatment of PD.

Acknowledgements

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References

Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F (1973) Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci 20: 415–455

Bethlem J, Den Hartog Jager WA (1960) The incidence and characteristics of Lewy bodies in idiopathic paralysis agitans (Parkinson's disease). J Neurol Neurosurg Psychiatry 23: 74–80

Birkmayer W (1969) The importance of monoamine metabolism for the pathology of the extrapyramidal system. J Neurovasc Relat 31 [Suppl 9]: 297+

Brannan T, Prikhojan A, Yahr MD (1998) Effects of repeated administration of L-DOPA and apomorphine on circling behavior and striatal dopamine formation. Brain Res 784: 148–153

Celesia GG, Wanamaker WM (1976) L-dopa-carbidopa: combined therapy for the treatment of Parkinson's disease. Dis Nerv Syst 37: 123–125

De Belleroche, Bradford HF (1976) Transport of amino acids and catecholamines in relation to metabolism and transmission. Adv Exp Med Biol 69: 395–404

De Jong GJ, Meerwaldt JD, Schmitz PIM (1987) Factors that influence the occurance of response variations in Parkinson's disease. Ann Neurol 22: 4–7

DiRocco A, Molinari SP, Kollmeier B, Yahr MD (1996) Parkinson's disease: progression and mortality in the L-DOPA era. Adv Neurol 69: 3–11

Fahn S (1983) Long-term treatment of tardive dyskinesia with presynaptically acting dopamine-depleting agents. Adv Neurol 37: 267–276

Fahn S, Libsch LR, Cutler RW (1971) Monoamines in the human neostriatum: topographic distribution in normals and in Parkinson's disease and their role in akinesia, rigidity, chorea and tremor. J Neurol Sci 14: 427–455

Fekete MI, Szentendrei T, Herman JP, Kanyicska B (1980) Effects of reserpine and antidepressants on dopamine and DOPAC (3,4-dihydroxyphenylacetic acid) concentrations in the striatum, olfactory tubercle and median eminence of rats. Eur J Pharmacol 64: 231–238

Ghosh PK, Hrdina PD (1977) Effects of tricyclic antidepressants on the content and metabolism of dopamine in the rat striatum. Can J Physiol Pharmacol 55: 383–388

Hefti F, Melamed E, Wurtman RJ (1980) Partial lesions of the dopaminergic system in rat brain: biochemical characterization. Brain Res 195: 123–137

Hoehn MM, Yahr MD (1967) Parkinsonism: onset, progression and mortality. Neurology 17: 427–442

Hornykiewicz O (1963) The topical localization and content of noradrenalin and dopamine (3-hydroxytyramine) in the substantia nigra of normal persons and patients with Parkinson's disease. Wien Klin Wochenschr 75: 309–312

Jankovic J (2000) Complications and limitations of drug therapy for Parkinson's disease. Neurology 55 [12 Suppl 6]: S2–S6

Koller WC (1996) Management of motor fluctuations in Parkinson's disease. Eur Neurol 36 [Suppl 1]: 43–48

Malamed E, Zoldan J, Galili-Mosberg R, Ziv I, Djaldetti R (1999) Current management of motor fluctuations in patients with advanced Parkinson's disease. J Neural Transm [Suppl 56]: 173–183

Martignoni E, Riboldazzi G, Calandrella D, Riva N (2003) Motor complications of Parkinson's disease. Neurol Sci 24 [Suppl 1]: S27–S29

Murata M, Horiuchi E, Kanazawa I (2001) Zonisamide has beneficial effects on Parkinson's disease patients. Neurosci Res 41: 397–399

Murata M, Horiuchi E, Tsuji S, Kanazawa I (2003) Zonisamide – a new drug for Parkinson's disease. 1. Long-term clinical effects. Neurology 60 [Suppl 1]: A288–A289

Nutt JG (1990) Levodopa-induced dyskinesias. Neurology 40: 340–345

Nutt JG, Woodward Wr, Carter JH, Gancher ST (1992) Effect of long-term therapy on the pharmacodynamics of levodopa. Arch Neurol 49: 1123–1130

Okada M, Kaneko S, Hirano T, Mizuno K, Kondo T, Otani K, Fukushima Y (1995) Effects of zonisamide on dopaminergic system. Epilepsy Res 22: 193–205

Olanow CW (2003) Present and future directions in the management of motor complications in patients with advanced PD. Neurology 61 [Suppl 3]: S24–S33

Sourkes TL (1972) Central actions of dopa and dopamine. Rev Can Biol 31 [Suppl]: 153–168

Stocchi F, Nordera G, Marsden CD (1997) Strategies for treating patients with advanced Parkinson's disease with disastrous fluctuations and dyskinesias. Clin Neuropharmacol 20: 95–115

Tolwani RJ, Jakowec MW, Petzinger GM, Green S, Waggie K (1999) Experimental models of Parkinson's disease: insights from many models. Lab Anim Sci 49: 363–371

Ungerstedt U (1971) Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol Scand 367 [Suppl]: 69–91

Van Laar T (2003) Levodopa-induced response fluctuations in patients with Parkinson's disease: strategies for management. CNS Drugs 17: 475–489

Von Voightlander PF, Moore KE (1971) Dopamine: release from the brain in-vivo by amantidine. Science 174: 408–409

Yahr MD, Duvoisin RC, Hoehn MM, Schear MJ, Barrett RE (1968) L-dopa (L-3,4-dihydroxyphenylalanine)-its clinical effects in parkinsonism. Trans Am Neurol Assoc 93: 56–63

Yahr MD, Duvoisin RC, Schear MJ, Barrett RE, Hoehn MM (1969) Treatment of parkinsonism with levodopa. Arch Neurol 21: 343–354

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Addiction: Part I. Benzodiazepines--Side Effects, Abuse Risk and Alternatives

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Benzodiazepines are widely prescribed for a variety of conditions, particularly anxiety and insomnia. They are relatively safe and, with overdose, rarely result in death. However, used chronically, benzodiazepines can be addicting. These agents are often taken in combination with other drugs of abuse by patients with addiction disorders. In such patients, alternatives to benzodiazepines may be preferable and may include antidepressants, anticonvulsants, buspirone, antihypertensive agents and the newer neuroleptic medications. Caution must be used when prescribing benzodiazepines to patients with a current or remote history of substance abuse. (Am Fam Physician 2000; 61:2121-8.)

There is little doubt of the therapeutic efficacy of benzodiazepines in reducing anxiety, inducing sleep and quelling panic symptoms. As noted in a 1990 report by the American Psychiatric Association (APA) on benzodiazepine dependence, toxicity and abuse,[1] the anxiolytic and hypnotic efficacy of benzodiazepines has been well established by numerous placebo-controlled studies.

Benzodiazepines are widely prescribed, with four of them--alprazolam (Xanax), clonazepam (Klonopin), diazepam (Valium) and lorazepam (Ativan)--listed among the top 100 most commonly prescribed medications.[2] Benzodiazepines generally produce almost immediate effects, and thus may be prescribed for short-term, intermittent, "as-needed" use. Because many of the anxiety disorders wax and wane over time, patients with these disorders often prefer benzodiazepines because these agents can be taken intermittently, when patients feel the need to take them, and most patients can use benzodiazepines judiciously.[1]

Benzodiazepines are also widely prescribed for other reasons, such as muscle spasticity, convulsive disorders, presurgical sedation, involuntary movement disorders, detoxification from alcohol and other substances, and anxiety associated with cardiovascular or gastrointestinal conditions[3] *(Table 1)*.

According to the APA report on benzodiazepines,[1] 11 to 15 percent of the adult population has taken a benzodiazepine one or more times during the preceding year, but only 1 to 2 percent have taken benzodiazepines daily for 12 months or longer. In psychiatric treatment settings and in substance-abuse populations, however, the prevalence of benzodiazepine use, abuse and dependence is substantially higher than that in the general population.[4,5]

Because benzodiazepines are controlled substances with abuse potential, special attention must be directed toward the patient's addiction history before these agents are prescribed. An understanding of the toxicity and side effects of benzodiazepines, abuse patterns and alternative anxiolytic and hypnotic agents may help clinicians maximize treatment outcomes and reduce medicolegal liability risks.

TABLE 1
Clinical Uses of Benzodiazepines

- Anxiety disorders
  - Acute anxiety
  - Generalized anxiety disorder
  - Panic disorder
  - Phobias (social, simple)
  - Post-traumatic stress disorder
  - Obsessive-compulsive disorder
- Insomnia
- Anxiety associated with medical illness
  - Cardiovascular
  - Gastrointestinal
  - Somatoform disorder
- Convulsive disorders
- Acute status epilepticus
  - Neonatal seizures or febrile convulsions
  - Preeclampsia
  - Tetanus
- Adjunct to other anticonvulsants
- Amnestic (before surgery or procedure)
- Spastic disorders and other types of acute muscle spasm
  - Cerebral palsy
  - Multiple sclerosis
  - Paraplegia secondary to spinal trauma
- Involuntary movement disorders
  - Restless leg syndrome
  - Akathisia associated with neuroleptic use
  - Choreiform disorders
  - Myoclonus
- Detoxification from alcohol and other substances
- Agitation or anxiety associated with other psychiatric conditions
  - Acute mania
  - Psychotic illness
  - Anxiety associated with depression
  - Impulse control disorders
  - Catatonia or mutism
- Other adjunctive uses
  - Surgery
  - Dentistry
  - Diagnostic studies, such as computed tomography, magnetic resonance imaging and endoscopy
  - Cardioversion
  - Chemotherapy

Information from Hollister L, Muller-Oerlinghausen B, Rickels K, Shader R. Clinical uses of benzodiazepines. J Clin Psychopharmacol 1993;13(suppl 1):1-169.

Neurochemistry

Benzodiazepine receptors are ubiquitous throughout the central nervous system. Benzodiazepine receptors are linked predominantly to g amino butyric acid (GABA) receptors, which sensitize benzodiazepine receptors to the neurotransmitter GABA, the most prominent inhibitory neurotransmitter in the central nervous system. Benzodiazepines enhance the affinity of the recognition site for GABA by inducing conformational changes that make GABA binding more efficacious. Activation of the benzodiazepine-GABA-chloride ionophor complex is responsible for producing the therapeutic anxiolytic effects of benzodiazepines and for mediating many of the side effects and, possibly, dependence and withdrawal from these drugs.[6]

Similarly, other sites for drug and neurotransmitter binding are associated with the GABA receptor complex, which serves as a primary site of action of benzodiazepines, barbiturates and other sedative-hypnotics, such as alcohol.[6] Benzodiazepines and barbiturates act at separate binding sites on the receptor to potentiate the inhibitory action of GABA. They do so by allosterically altering the receptor (changing its conformation) so that it has a greater binding affinity for GABA. Ethanol modifies the receptor by altering the membrane environment so that it has increased affinity for GABA and the other sedative-hypnotic drugs. That benzodiazepines, barbiturates and ethanol all have related actions on a common receptor type, which explains their pharmacologic synergy and cross tolerance. Thus, benzodiazepines are used during alcohol detoxification.

With long-term high-dose use of benzodiazepines (or ethanol), there is an apparent decrease in the efficacy of GABA-A receptors, presumably a mechanism of tolerance.[6,7] When high-dose benzodiazepines or ethanol are abruptly discontinued, this "down-regulated" state of inhibitory transmission is unmasked, leading to characteristic withdrawal symptoms such as anxiety, insomnia, autonomic hyperactivity and, possibly, seizures.

Toxicity and Side Effects

With the introduction of chlordiazepoxide (Librium) in 1960, and because of the relative safety of benzodiazepines, these agents rapidly replaced barbiturates as sedative-hypnotics. They cause significantly less respiratory depression than barbiturates and, consequently, are rarely lethal in an overdose.

Fatal overdose with benzodiazepines is rare. When it does occur, the combination of benzodiazepines and alcohol, with or without opiates, is often the cause of death.

As a class of drugs, benzodiazepines share many clinical properties, although the different agents in this class may display different pharmacokinetic and pharmacodynamic properties *(Table 2)*. Pharmacologic properties such as potency, half-life and lipophilicity, the duration of treatment and the rate of a dosage increase or decrease have a bearing on the occurrence of side effects.[1] The development of physiologic dependence is somewhat predictable and is proportional to the total benzodiazepine exposure (dose 3 duration of treatment), although significant variability may exist among patients.

Toxicity and Drug Interactions

When used alone, benzodiazepines carry an extremely low risk of acute toxicity. However, benzodiazepines often are used

with other types of medications, including other drugs with abuse potential, and these drugs can enhance the toxic effects of benzodiazepines. The latter interact synergistically with other central nervous system depressants, including other hypnotics, sedating antidepressants, neuroleptics, anticonvulsants, antihistamines and alcohol.[8] Fatal overdoses in addicted patients often involve the combination of benzodiazepines and alcohol, with or without opiates. In addition, pharmacokinetic drug interactions may occur. For instance, selective serotonin reuptake inhibitors (SSRIs) may increase diazepam blood levels,[9] and nefazadone (Serzone) may increase alprazolam levels[10] through hepatic enzyme inhibition, leading to increased sedative-hypnotic effects or side effects.

TABLE 2
Potency and Half-Life of Various Benzodiazepines

High-potency benzodiazepines
Drugs with a short half-life
- Alprazolam (Xanax)
- Lorazepam (Ativan)
- Triazolam (Halcion)

Drugs with a long half-life
- Clonazepam (Klonopin)

Low-potency benzodiazepines
Drugs with a short half-life
- Oxazepam (Serax)
- Temazepam (Restoril)

Drugs with a long half-life
- Chlordiazepoxide (Librium)
- Clorazepate (Tranxene)
- Diazepam (Valium)
- Flurazepam (Dalmane)

Psychomotor Retardation
Psychomotor slowing may be especially profound following initial administration of a benzodiazepine or with a sudden dosage increase. It also may be noted in patients, such as the elderly, who have decreased rates of metabolism or greater susceptibility to central nervous system depression.[8] Psychomotor symptoms include drowsiness, poor concentration, ataxia, dysarthria, motor incoordination, diplopia, muscle weakness, vertigo and mental confusion.[11] Studies of the psychomotor effects suggest that benzodiazepines slow reaction time and impair driving skills, increasing the risk of motor vehicle crashes in patients who are taking these agents.[12]

Memory Impairment
Benzodiazepines induce anterograde amnesia, which accounts for the beneficial effects of benzodiazepines such as midazolam (Versed) for presurgical medication. These specific amnestic effects appear to be separate from sedation.[11] Episodic memory (the remembering of recent events and the circumstances in which they occurred and their time sequences) is particularly impaired and more markedly so in heavy alcohol drinkers who also use benzodiazepines. Specific deficits in visuospatial ability and sustained attention have also been described in patients who have taken therapeutic doses of benzodiazepines regularly for longer than one year.[13]

Paradoxical Disinhibition
Increased excitement, irritability, aggression, hostility and impulsivity may occur in some patients who take benzodiazepines. This paradoxical disinhibition may, in rare cases, result in attacks of rage or violence, or other indiscretionary or antisocial behaviors.[14] Such reactions may be due to disinhibition of behavioral tendencies normally suppressed by social restraints (as can also be the case with alcohol). These reactions occur most commonly in children, in the elderly and in persons with developmental disabilities.

Depression and Emotional Blunting
An association has been noted between benzodiazepine use and depressive symptoms and,

in some cases, the emergence of suicidal ideation. Some evidence indicates that higher benzodiazepine dosages are associated with an increased risk of depression and that reducing the dosage or discontinuing therapy may resolve the depressive symptoms.[15] Although the mechanism of this action is unclear, benzodiazepine-related depression might occur as a physiologic result of a reduction in central monoamine activity.

"Emotional anesthesia" may also be seen in clinical practice. This effect may be sought by drug addicts who become progressively more incapable of tolerating their emotions and life stressors.

Adverse Effects in Pregnancy

Benzodiazepines cross the placenta and are classified as class D teratogens. They may lead to the development of dependence and consequent withdrawal symptoms in the fetus.[16] Benzodiazepines are excreted in breast milk and thus are usually contraindicated in breast-feeding mothers.

Tolerance

Tolerance to all of the actions of benzodiazepines can develop, although at variable rates and to different degrees. Tolerance to the hypnotic effects tends to develop rapidly, which may be beneficial in daytime anxiolysis but makes long-term management of insomnia difficult.[17] Patients typically notice relief of insomnia initially, followed by a gradual loss of efficacy.[18] Tolerance to the anxiolytic effect seems to develop more slowly than does tolerance to the hypnotic effects, but there is little evidence to indicate that benzodiazepines retain their efficacy after four to six months of regular use.[19,20] Benzodiazepine therapy is often continued to suppress withdrawal states, which usually mimic symptoms of anxiety. Dosage escalation often maintains the cycle of tolerance and dependence, and patients may have difficulty discontinuing drug therapy.

Dependence

Benzodiazepine therapy can give rise to physiologic and psychologic dependence based on the drug's dosage, duration of therapy and potency.[1] Thus, dependence will develop sooner (such as in one to two months) in a patient who is taking a high dosage of a high-potency agent such as alprazolam than in a patient who is receiving a relatively low dosage of a long-acting, low-potency agent such as chlordiazepoxide. As a result of physiologic dependence, withdrawal symptoms emerge with rapid dose reduction or abrupt discontinuation of the drug.

Short-acting, high-potency agents, such as alprazolam, cause dependence sooner than longer-acting agents such as chlordiazepoxide and diazepam.

Psychologically, long-term use of benzodiazepines may lead to overreliance on the need for the agent, loss of self-confidence and varying degrees of drug-seeking behavior.[8] Patients may be reluctant to discontinue the drug because of misplaced fears or anticipatory anxiety. Some patients combine alcohol with benzodiazepines when they are not able to acquire the desired or "needed" effects.

Short-Term Withdrawal Symptoms

Withdrawal effects from therapeutic dosages of benzodiazepines are mainly anxiety symptoms.[1,21] In addition, autonomic instability (i.e., increased heart rate and blood pressure level, tremulousness, diaphoresis), insomnia and sensory hypersensitivity are common. The most serious acute withdrawal symptoms are seizures and delirium tremens, which most commonly occur with abrupt discontinuation. The time frame for the emergence of acute

withdrawal symptoms corresponds to the half-life of the particular agent being used.

Some elements of withdrawal are believed to occur in a majority of patients who have taken therapeutic dosages of benzodiazepines for more than a few months, although the severity of withdrawal symptoms generally depends on the amount of the original dosage, the rate at which the dosage is tapered, the selection of patients and the definition of withdrawal symptoms.[1,18]

Protracted Withdrawal

A protracted abstinence syndrome has been observed by addictionologists who are familiar with benzodiazepine addiction.[22] Symptoms include prolonged (for several months) anxiety, depression and insomnia. In addition, physical symptoms related to gastrointestinal, neurologic and musculoskeletal effects may occur. This abstinence phenomenon may develop despite long, slow, judicious tapering of the dosage and is hypothesized to result from chronic neuroadaptation.

Effects in Elderly Patients

Among the elderly, the risk of drug interactions, psychomotor slowing, cognitive dysfunction and paradoxical disinhibition may be amplified. Benzodiazepine use in the elderly is associated with an increased rate of falls that cause hip and femur fractures and an increased likelihood of motor vehicle crashes.[23,24] Cognitive impairment is common, although memory impairment may be reversible when benzodiazepines are discontinued.[25]

Cognitive deterioration associated with normal aging processes and dementia can be worsened by benzodiazepine side effects. Cortical suppression mechanisms may be disturbed in the elderly, and disinhibited behaviors may increase with benzodiazepine use. With less cognitive and social reserve in the elderly patient, the short- and long-term withdrawal symptoms and other benzodiazepine side effects may lead the patient to frequently visit or telephone the physician. The physician may feel "trapped" into arguing against the use of benzodiazepines and prescribing benzodiazepines to elderly patients. In one study,[26] this impasse was broken by referring elderly patients to inpatient detoxification, which resulted in a dramatic decrease in annual physician visits.

Benzodiazepine Abuse

Benzodiazepines are rarely the preferred or sole drug of abuse. An estimated 80 percent of benzodiazepine abuse is part of polydrug abuse, most commonly with opioids.[27] A two-year treatment outcome study by the National Institute on Drug Abuse[28] found that 15 percent of heroin users also used benzodiazepines daily for more than one year, and 73 percent used benzodiazepines more often than weekly. Studies indicate that from 5 percent to as many as 90 percent of methadone users are also regular users of benzodiazepines. High-dose benzodiazepine abuse is especially prevalent in patients who are taking methadone.[29]

Studies indicate that 3 to 41 percent of alcoholic persons report that they abused benzodiazepines at some time, often to modulate intoxication or withdrawal effects.[4] The contemporary alcoholic is usually a multiple-drug user. As many as 80 percent of alcoholics under the age of 30 have been addicted to or use at least one other drug.[27]

Medical prescriptions constitute the primary source of supply for people who abuse benzodiazepines. Prescriptions may also have a street value, which encourages rerouting to

illicit sources. Benzodiazepines have multiple uses for polydrug addicts: they are used to enhance the euphoriant effects of opioids (such as to "boost" methadone doses), to alleviate withdrawal or abstinence syndromes (such as between heroin "fixes"), to temper cocaine highs, to augment alcohol synergistically and to modulate withdrawal states.

As potential drugs of abuse, short-acting benzodiazepines seem to be preferred among addicts because of the rapidity of their onset of action.[30] In general, mood-altering substances are most highly reinforcing in patients with chemical dependence if the agent has a rapid onset of action, a high potency, a brief duration of action, high purity and water solubility (for intravenous use) or high volatility (ability to vaporize if smoked).[31] Data suggest that highly lipophilic benzodiazepines (for example, those that cross the blood-brain barrier more rapidly), such as diazepam, and agents with a short half-life and high potency, such as lorazepam or alprazolam, are the most reinforcing benzodiazepines and, therefore, the ones most likely to be associated with abuse.[30]

Clonazepam is a high-potency benzodiazepine with a long half-life. It is widely prescribed for a variety of psychiatric and neurologic conditions. Although clonazepam is perceived as "safe," addiction medicine specialists have found that it is also frequently abused as a street drug. On the other hand, oxazepam (Serax), clorazepate (Tranxene) and chlordiazepoxide appear to have lower reinforcing effects than other benzodiazepines.

Compared with generic formulations, trade-name prescription drugs can be worth twice as much per tablet when they are sold on the street because they are readily recognizable as the "real thing" when compared with the photographs of tablets in the *Physicians' Desk Reference.*[31] Generic pills are often unrecognizable and hence are worth less when diverted for street sale. In many U.S. cities, the street value of Xanax or Klonopin may be $5 to $10 per pill, depending on dosage strength.

Benzodiazepine Alternatives

The problems with benzodiazepine dependence, tolerance, withdrawal, rebound and abuse limit their use for long-term treatment of anxiety disorders in patients with alcohol or drug addiction. A growing body of literature now supports the anxiolytic efficacy of numerous other agents *(Table 3).* Antidepressants, anticonvulsants, buspirone (Buspar), certain antihypertensive agents and newer neuroleptics all have been shown to be effective in subsets of patients with anxiety.[32]

Most addiction medicine specialists believe that benzodiazepines are relatively contraindicated in patients with current alcohol or drug abuse problems and in patients who are in recovery. To choose an appropriate alternative to a benzodiazepine, physicians should be able to delineate which subtype of anxiety disorder exists in a particular patient. Patients should be encouraged to understand that the onset of action of antidepressants, buspirone and anticonvulsants is not as immediate as that of benzodiazepines. Therapy may require patience and, because of side effects, a low dosage may be required initially.

TABLE 2
Efficacy of Pharmacologic Agents in the Treatment of Anxiety Disorders

Disorder	BZs	SSRIs	TCAs	ACVs*	Bu	ANs†	AHTs‡
Acute anxiety	++					+	+
Generalized anxiety disorder	++	+	++	±	++		
Panic disorder	++	++	++	+			
Social phobia	+	++	+		+		
Post-traumatic stress disorder	±	+	+	+	+	+	
Obsessive-compulsive disorder		++	+		+	±	

BZs = benzodiazepines; SSRIs = selective serotonin reuptake inhibitors; TCAs = tricyclic antidepressants; ACVs = anticonvulsants; Bu = Buspirone (Buspar); ANs = atypical neuroleptics; AHTs = antihypertensives.
++ = proven efficacy in numerous controlled trials; + = reported efficacy in open trials or in patients with comorbid depression; ± = equivocal efficacy, anecdotal reports or adjunctive use; = no good clinical evidence of efficacy.
*--Anticonvulsants include valproic acid (Depakene) and gabapentin (Neurontin).
†--Atypical neuroleptics include risperidone (Risperdal), olanzapine (Zyprexa) and quetiapine (Seroquel).
‡--Antihypertensives include beta blockers and clonidine (Catapres).

Insomnia

Insomnia is a common sequela of numerous medical and psychiatric conditions, and is often associated with substance-use disorders, early abstinence or protracted withdrawal. Management of insomnia includes attention to sleep hygiene techniques, such as maintaining a regular sleep-wake cycle, avoiding daytime naps, avoiding caffeine or heavy meals at night, and engaging in gentle exercise or utilizing other relaxation techniques.

Nonbenzodiazepine pharmacotherapies for the management of insomnia include the sedating antidepressant trazodone (Desyrel), tertiary tricyclic antidepressants such as amitriptyline (Elavil) and doxepin (Sinequan), and newer antidepressant agents such as nefazodone and mirtazapine (Remeron).[33]

Zolpidem (Ambien), an imidazopyridine, is a hypnotic agent with a chemical structure unrelated to benzodiazepines.[34] Unlike the benzodiazepines, zolpidem does not interfere with sleep stages 3 and 4, nor does it decrease rapid-eye-movement (REM) sleep. Tolerance and withdrawal symptoms do not appear as readily with this agent as with benzodiazepines. However, zolpidem is classified as a schedule IV controlled substance (like benzodiazepines), and synergistic effects with benzodiazepines and alcohol have been observed. Problems with vivid dreams, nightmares and rebound insomnia have also been reported.[34]

Final Comment

Although benzodiazepines are effective in a wide range of medical and psychiatric conditions, caution must be exercised with their use, particularly when these agents are prescribed to patients with an active or remote history of substance abuse or addiction.

Their greatest asset is also their greatest liability: drugs that work immediately tend to be addictive. Compared with benzodiazepines, antidepressants have a longer onset of action but are the best agents for long-term treatment of anxiety disorders. Anticonvulsants, antipsychotics, antihypertensives and buspirone also are effective but have an intermediate onset of action.

Clinical judgment is based on an assessment of the risks versus the benefits of therapy. Such an approach might take into account whether substance abuse is active or remote, whether other family members or other health care professionals are actively involved in the patient's care, and how well the physician knows the patient. Physicians should also freely seek consultation from specialists such as psychiatrists and addiction medicine specialists. Education, consultation and documentation not only improve the level of clinical care but also provide necessary risk management and medicolegal liability protection.

This is Part I of a two-part article on addiction. Part II, "Identification and Management of the Drug-Seeking Patient," will appear in the next issue.

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REFERENCES

1. Salzman C, for Task Force on Benzodiazepine Dependency, American Psychiatric Association. Benzodiazepine dependence, toxicity, and abuse: a task force report of the American Psychiatric Association. Washington, D.C.: American Psychiatric Association, 1990.
2. American Druggist. Top 200 drugs of 1995. New York, N.Y.: Hearst Corp, 1996:18-26.
3. Hollister LE, Muller-Oerlinghausen B, Rickels K, Shader RI. Clinical uses of benzodiazepines. J Clin Psychopharmacol 1993;13(suppl 1):1S-169S.
4. Ciraulo DA, Sands BF, Shader RI. Critical review of liability for benzodiazepine abuse among alcoholics. Am J Psychiatry 1988;145:1501-6.
5. Busto UE, Romach MK, Sellers EM. Multiple drug use and psychiatric comorbidity in patients admitted to the hospital with severe benzodiazepine dependence. J Clin Psychopharmacol 1996;16:51-7.
6. Arana GW, Hyman SE. Handbook of psychiatric drug therapy. 2d ed. Boston: Little, Brown, 1991: 128-61.

7. Miller LG. Chronic benzodiazepine administration: from the patient to the gene. J Clin Pharmacol 1991;31:492-5.
8. Ashton H. Toxicity and adverse consequences of benzodiazepine use. Psychiatric Annals 1995;25:158-65.
9. Janicak PG, Davis JM, Preskhorn SH, Ayd FJ. Principles and practice of psychopharmacotherapy. Baltimore: Williams & Wilkins 1993:287.
10. Geene DS, Dockens RC, Salazar DE, et al. Coadministration of nefazadone and benzodiazepines. I. pharmacokinetic assessment [Abstract]. Clin Pharmacol Ther 1994;55:141.
11. Lader M. Long-term benzodiazepine use and psychological functioning. In: Freeman HL, Rue Y, eds. The benzodiazepines in current clinical practice. International congress and symposium series: proceedings of a symposium sponsored by Wyeth Laboratories. London: Royal Society of Medicine Services, 1987:55-69.
12. Barbone F, McMahon AD, Davey PG, Morris AD, Reid IC, McDevitt DC, et al. Association of road-traffic accidents with benzodiazepine use. Lancet 1998;352:1331-6.
13. Curran V. Memory functions, alertness, and mood of long-term benzodiazepine users: a preliminary investigation of the effects of normal daily dose. J Psychopharmacol 1992;6:69-75.
14. van der Bijl P, Roelofse JA. Disinhibitory reactions to benzodiazepines: a review. J Oral Maxillofac Surg 1991;49:519-23.
15. Smith BD, Salzman C. Do benzodiazepines cause depression? Hosp Comm Psychiatry 1991;42: 1101-2.
16. Bergman U, Rosa FW, Baum C, Wiholm BE, Faich GA. Effects of exposure to benzodiazepines during fetal life. Lancet 1992;340:694-7.
17. Kales A, Scharf MB, Kales JD. Rebound insomnia: a new clinical syndrome. Science 1978;201:1039-41.
18. Schneider-Helmert D. Why low-dose benzodiazepine-dependent insomniacs can't escape their sleeping pills. Acta Psychiatr Scand 1988;78:706-11.
19. Mellinger GD, Balter MB, Uhlenhuth EH. Prevalence and correlates of the long-term use of anxiolytics. JAMA 1984;251:375-9.
20. Marriott S, Tyrer P. Benzodiazepine dependence. Avoidance and withdrawal. Drug Saf 1993;9:93-103.
21. Busto U, Sellars EM, Naranjo CA, Cappell H, Sanchez-Craig M, Sykora K. Withdrawal reaction after long-term therapeutic use of benzodiazepines. N Engl J Med 1986;315:854-9.
22. Ashton H. Protracted withdrawal syndromes from benzodiazepines. J Subst Abuse Treat 1991;8:19-28.
23. Herings RM, Stricker BH, de Boer A, Bakker A, Sturmans F. Benzodiazepines and the risk of falling leading to femur fractures. Dosage more important than elimination half-life. Arch Intern Med 1995; 55:1801-7.
24. Hemmelgarn B, Suissa S, Huang A, Boivin IF, Pinard G. Benzodiazepine use and the risk of motor vehicle crash in the elderly. JAMA 1997;278:27-31.
25. Salzman C, Fisher J, Nobelk, Glassman R, Wolfson A, Kelly M. Cognitive improvement following benzodiazepine discontinuation in elderly nursing home residents. Int J Geriatr Psychiatry 1992;7:89-93.
26. Burke KC, Meek WJ, Krych R, Nisbett R, et al. Medical services use by patients before and after detoxification from benzodiazepine dependence. Psychiatric Services 1995;46(2):157-60.
27. Gold MS, Miller NS, Stennie K, Populla-Vardi C. Epidemology of benzodiazepine use and dependence. Psychiatric Annals 1995;25:146-8.
28. Dumont RL. Abuse of benzodiazepines--the problems and the solutions. A report of a Committee of the Institute for Behavior and Health, Inc. Am J Drug Alcohol Abuse 1988;14(suppl 1):1-69.
29. Iguchi MY, Griffiths RR, Bickel WK, et al. Relative abuse liability of benzodiazepines in methadone-maintained populations in three cities. In: Harris LS, ed. Problems of drug dependence, 1988: proceedings of the 50th Annual Scientific Meeting, the Committee on Problems of Drug Dependence, Inc. Rockville, Md.: U.S. Department of Health and Human Services, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute on Drug Abuse, Office of Science, 1989. DHHS publication no. (ADM) 89-1605.
30. Roache JD, Meisch RA. Findings from self-administration research on the addiction potential of benzodiazepines. Psychiatric Annals 1995;25(3):153-7.
31. Parran TV. Prescription drug abuse. A question of balance. Med Clin North Am 1997;81:967-78.
32. Longo LP. Non-benzodiazepine pharmacotherapy of anxiety and panic in substance abusing patients. Psychiatric Annals 1998;28(3):142-53.

33. Longo LP, Johnson B. Treatment of insomnia in substance abusing patients. Psychiatric Annals 1998; 28(3):154-9.
34. Zolpidem for insomnia. Med Lett Drugs Ther 1993; 35:35-6.

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